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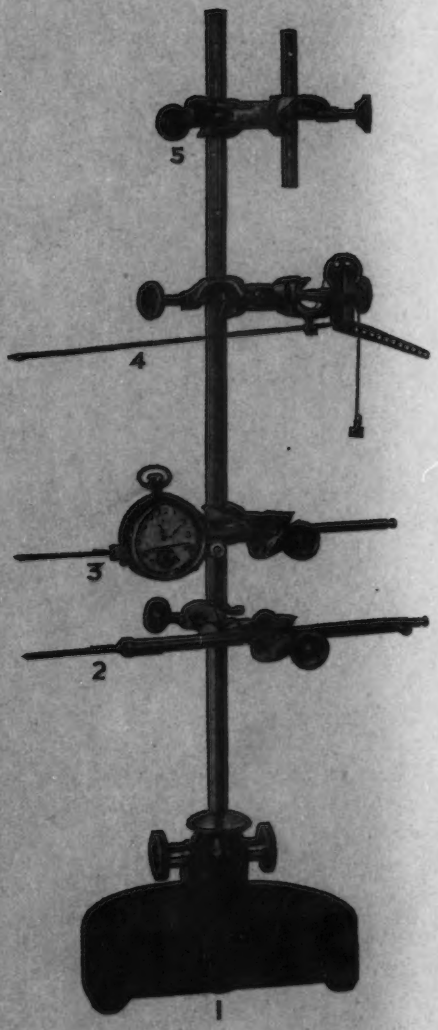

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VOL. 77 JULY 1, 1926 No. 2

FURTHER EVIDENCE THAT SMALL QUANTITIES OF COPPER, MANGANESE AND ZINC ARE FACTORS IN THE METABOLISM OF ANIMALS

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Contribution from the laboratory of chemical research of the Kentucky Agricultural Experiment Station, Lexington

Received for publication February 22, 1926

In previous reports (1), (2), (3) the author has called attention to the fact that small quantities of copper, manganese, zinc, nickel, boron and arsenic are widely distributed in natural foods and in many instances compounds of these elements occur in larger proportions in the tissues of those organs of plants and animals which have the greatest vitamin potency. This very interesting association of small quantities of a number of elements which have hitherto been regarded as having no particular function in the processes of either plant or animal metabolism suggests the possibility of a much greater significance than mere accidental occurrence. The necessity for copper, manganese and zinc and the part played by them in the nutrition of animals have long been questions upon which there is a lack of definite information.

The purpose of this paper is to present the results thus far obtained in an investigation the object of which is to ascertain whether or not the small quantities of copper, manganese and zinc which occur in natural foods have important physiological functions in the metabolism of animals. A point of fundamental importance to be kept in mind in obtaining evidence on this subject is the fact that no product of either vegetable or animal matter thus far examined has been found entirely free from copper, manganese or zinc. After having tested several samples of commercial casein and starch, such as are in common use in compounding synthetic rations for rats, the writer is convinced that unless these products have been subjected to an unusual degree of purification they invariably contain small amounts of manganese, copper and zinc which should not be

overlooked in a careful study of the mineral requirements in the metabolism of animals.

Another important point in the technic of experiments with rats on this subject is a cage from which they cannot obtain corrosion compounds or small bits of metal by gnawing at the wires of the cages that confine them.

These sources of contamination from compounds of the metals under investigation are most difficult to control and are, in the mind of the writer, important factors which have not been taken into serious consideration in nutritional experiments heretofore.

EXPERIMENTAL WORK. For the experiments herein described a special type of glass cage was constructed by fitting large glass bell-jars with monel metal wire gauze and sheet aluminum in the way shown in the accompanying photographs (fig. 1).

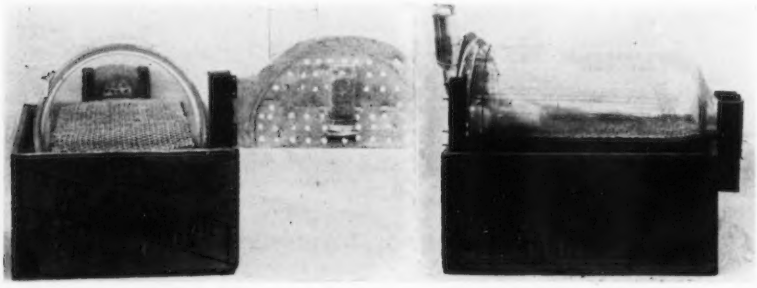

Fig. 1. Photographs of improvised rat cages; left, front view; right, side view

The self-feeder shown attached to the inside of the door of the cage on the left was discarded for the more convenient form described in the text.

The bell-jars were supported on their sides in boxes of the proper size which were obtained at a grocery store. The wire gauze was cut and bent to fit the walls of the bell-jar and formed a floor which was made fast to the box at each end of the bell-jar. The perforated sheet aluminum door was fashioned, fitted and attached by means of hinges to a wood upright fastened to the box at the front end. The glass containers for the food and water were attached to the door of the cage. By this arrangement the rats were in contact with glass, aluminum and monel metal. Monel metal gauze was used because of its non-corrosive properties.

The ration. The ration was made from the following substances: Argo corn starch 54 per cent, commercial casein 26 per cent, domino cane sugar 10 per cent, Price's lard 5 per cent, mineral mixture 5. The mineral mixture was made from the following chemical compounds which were of a high grade of purity, NaCl 46, $MgSO_4$, $7H_2O$ 71, $NaH_2PO_4H_2O$ 92, K_2HPO_4 254, $Ca(C_3H_5O_3)_25H_2O$ 347, ferric citrate 31, NaF 1, KI 5. Enough starch, casein, sugar, lard and mineral mixture to supply food for about

10 days were weighed out in the dry state and transferred to a graniteware dishpan and enough distilled water added to make a moist dough. After thoroughly mixing the components of the ration, equal and separate portions of the dough were weighed out and further mixed with enough of the respective compounds of copper, manganese or zinc to make the moisture-free ration to contain about 25 parts per million of copper, 100 parts per million of manganese and 25 parts per million of zinc, respectively. In the experiment where each of these compounds was added to the ration the same proportions were maintained. Manganese peptonate was the compound used to supply manganese, copper sulfate was the compound used to supply copper, and zinc lactate was the compound used to supply zinc. After each of the mineral compounds being studied was added to and thoroughly mixed with the respective portions of the ration the mixtures were transferred to aluminium baking pans and dried in a gas oven at a temperature near 100°C. until most of the added water was removed, after which the food was ground, and placed in clean glass jars from which portions were placed in the self-feeding apparatus attached to each cage.

The self-feeders were made by drawing out and bending a wide glass tube and blowing a hole of a sufficient size and at the proper point so that the rats could procure their food in small bits without contaminating it with their excreta or wasting it unduly by scratching and dropping it through the wire-gauze floor, as is the case when food is placed in open containers. Small squares cut from window glass were kept beneath the self-feeders to catch bits of food that would otherwise have fallen through the gauze floor. The self-feeders also caused a certain amount of exercise to be taken by the rats during feeding because the hole in the self-feeder was made just large enough to allow only one rat to procure food at a time. This arrangement resulted in a scramble among the rats while feeding and especially when fresh portions of the rations were placed in the self-feeders.

Four rats were kept in each cage and were distinguished from each other by the number of small holes punched in the ears. The rats were weighed separately once each week. The wire-gauze floor could be easily removed and the cages cleaned as often as necessary. Sawdust was kept beneath the wire-gauze, out of reach of the rats, and served as an absorbent for the urine. The small end of each bell-jar was fitted with a perforated aluminum cap which permitted the circulation of air through the cages.

The experiments were started on May 1, 1925, and continued for 17 weeks. The rats that survived at the end of the 17th week were chloroformed and the carcasses of each lot were combined into one sample for analysis for mineral constituents. Nearly all the rats had attained their maximum weight by July 1, the end of the 9th week. During that month they declined rapidly in weight and those in the check experiments were among the first to succumb followed by those that received

the zinc compound, and these, in turn, by those that received copper. The rats that received manganese, both alone and in combination with copper and zinc, survived the longest and were chloroformed after most of the rats in the other experiments had died. There was no cannibalism in any of the experiments.

Some of the rats in all the experiments developed ophthalmia but not all of them. The ones that did develop sore eyes did so in the last week or so of their existence. The author is inclined to believe that the ophthalmia was the result of an excess of minerals in the ration. According to the recent findings of McCollum (4), a synthetic ration containing 5 per cent of minerals will produce sore eyes when fat soluble A is present in ample amount.

TABLE 1

Increase in weight of the rats during the experiment

CAGE NUMBER	CAGE NO. 1, CHECK				CAGE NO. 2 + Mn				CAGE NO. 3 + Cu			
Rat number	1	2	3	4	1	2	3	4	1	2	3	4
Maximum weight attained	139	135	133	121	171	143	143	146	156	138	152	123
Initial weight	73	79	75	62	63	64	61	56	66	64	77	56
Gain	66	56	58	59	108	79	82	90	90	74	75	67
Average gain	59.75 grams				89.75 grams				76.5 grams			

CAGE NUMBER	CAGE NO. 4 + Zn				CAGE NO. 5 + Mn, Cu, Zn		
Rat number	1	2	3	4	1	2	3
Maximum weight attained	144	138	127	116	137	167	145
Initial weight	65	64	48	43	65	43	65
Gain	79	74	79	73	72	124	80
Average gain	76.25 grams				92.0 grams		

The increase in weight of the rats is shown in table 1 and in the graphs which follow.

From table 1 and the graphs it is to be observed that the rats in cage 1, the checks, made the least growth of any, although their average initial weight was considerably more than the average initial weight of the rats in any of the other experiments. Moreover, the rats receiving manganese alone, cage 2, and manganese in combination with copper and zinc, cage 5, made the largest average gains. The author considers this a point of considerable significance in obtaining evidence of the necessity of the elements under consideration in the metabolism of animals. It should be noted, however, that cage 5 contained only three rats and that one of these made an exceptionally large gain in weight, the gains of the other

two being about the same as those of the best two in the zinc cage. One rat in the manganese cage, also, made an exceptionally large gain in weight, the gains of the other three being about the same as the two poorest in cage 5. These considerations render the averages for these cages less significant than if the gains of the individuals had been consistent.

The rats that survived until the 17th week were chloroformed, the intestinal tract removed and the carcasses weighed and dried at 100°C. Those in the experiments which had died previous to this date had been treated in a like manner for chemical analysis. The carcasses of the rats in each experiment were combined into one sample and analyzed separately for the various mineral constituents. The results were calculated to the moisture free basis and also to the basis of the maximum live weight obtained. Five other normal rats, one a mature male about two years old and four rats of one litter, two males and two females, which were 75 days old, were chloroformed and the intestinal tract removed. The two carcasses of the male rats were combined for one sample and the

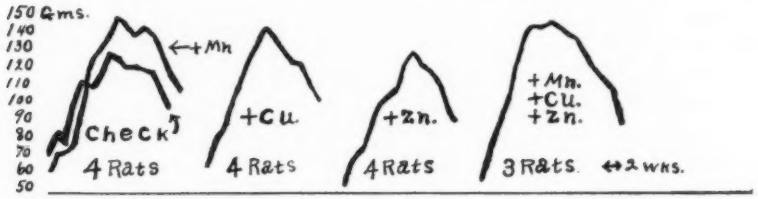

Fig. 2

two female carcasses combined for another sample. The two samples were dried at 100°C. and analyzed separately. The analysis represents the mineral content of normal male and female rats of the same litter that were 75 days old. Twelve normal rat pups which were less than 6 hours old were chloroformed and dried at 100°C. and analyzed for the mineral matter contained in their carcasses at this stage of their life.

Method of analysis of rat carcasses. The method used for destroying the organic matter contained in the carcasses of the rats and obtaining the mineral matter in solution without loss was as follows:

The dry carcasses were broken up in a wedgewood mortar and transferred to a 2-liter, round-bottom, pyrex flask. About 25 cc. of pure concentrated sulfuric acid per 100 grams of material were added and the contents of the flask were agitated until the acid was absorbed. The flask was placed over a hole on a hot water bath in a hood and 10 cc. portions of pure concentrated nitric acid added from time to time until the contents of the flask were in a semi-liquid condition. After the fumes of nictric acid had been expelled the contents of the flask were transferred to

TABLE 2

	MINERAL MATTER CONTAINED IN THE MOISTURE-FREE CARCASSES OF WHITE ALBINO RATS FED A SYNTHETIC RATION TO WHICH WERE ADDED SMALL AMOUNTS OF COMPOUNDS OF MANGANESE, COPPER AND ZINC. DIGESTIVE TRACT EXCLUDED					MINERAL MATTER CONTAINED IN THE MOISTURE-FREE CARCASSES OF NORMAL ALBINO RATS FED THE STEENBOCK RATION. DIGESTIVE TRACT EXCLUDED			
Cage numbers	1	2	3	4	5				
Number of rats analyzed	3	4	4	4	3	1 two-year old male	2 males 75 days old. Same litter as females	2 females 75 days old. Same litter as males	12 rat pups, 6 hours old
Treatment	Synthetic ration only	Synthetic ration + Mn	Synthetic ration + Cu	Synthetic ration + Zn	Synthetic ration + Mn, Cu, and Zn				
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Moisture, H_2O	67.25	64.40	67.29	68.50	69.15	67.27	69.22	68.57	83.74
Copper, Cu	0.0015	0.0013	0.0030	0.0011	0.0028	0.0012	0.0016	0.0021	0.0030
Iron, Fe	0.0235	0.0257	0.0272	0.0321	0.0378	0.0238	0.0271	0.0290	0.1162
Manganese, Mn	0.0003	0.0004	0.0003	0.0002	0.0003	0.0003	0.0003	0.0003	Present n.e.*
Zinc, Zn	0.0146	0.0160	0.0117	0.0293	0.0279	0.0113	0.0125	0.0151	Present n.e.*
Calcium, Ca	7.659	7.980	7.555	7.021	6.730	3.590	3.872	4.360	2.132
Magnesium, Mg	0.3255	0.2860	0.4293	0.2777	0.2354	0.170	0.181	0.188	0.222
Phosphorus, P	4.264	4.456	4.088	3.880	3.862	2.265	2.878	3.080	2.530
Potassium, K	1.170	0.8502	1.003	1.0838	1.026	0.950	1.192	1.140	1.608
Sodium, Na	1.148	0.7844	1.006	0.9926	1.016	0.742	0.893	0.929	1.933

*Sample too small.

a flat-bottom porcelain dish by means of hot distilled water after which the excess of water and sulfuric acid was expelled by cautiously heating on a sand bath until the contents of the dish had become a dry, hard mass. When cool, the dry residue was broken in pieces which were transferred to quartz dishes and burned at a low temperature in a muffle furnace to a white ash. Any matter that adhered to the sides of the procelain dishes was easily removed and absorbed on moist pieces of quantitative filter paper and ashed with the carbonized residue. The ash was dissolved in hot dilute hydrochloric acid, filtered, and the residue washed thoroughly. Copper was precipitated from the hot hydrochloric acid solution of the ash by passing hydrogen sulfide through the solution which was allowed to stand tightly stoppered over night. After filtering out the precipitate of copper sulfide and washing with a dilute solution of hydrochloric acid which had been saturated with hydrogen sulfide, the filtrate was oxidized by adding nitric acid and allowed to stand on the hot water bath for an hour or more. The filtrate was cooled and made to a definite volume from which aliquots were taken for the determination of the other mineral constituents. The copper was estimated by the colorimetric xanthate method (5), manganese by the colorimetric periodiate method (6) and zinc by the potassium ferrocyanide turbidity method (7). Calcium was precipitated by the McCrudden method (8) and the washed oxalate titrated with N/10 potassium permanganate. Phosphorus was determined volumetrically and potassium and sodium by the well-known chloroplatinate method.

Each lot of rat carcasses was analyzed by the above mentioned methods and gave results from which the figures in tables 2 and 3 were calculated.

Table 2 contains results which are of some interest and therefore require further explanations and comments. The table contains the results of analyses of the carcasses of albino rats which were fed a synthetic ration to which were added small amounts of compounds of manganese, copper and zinc, either separately or in combination. It also contains, for the purpose of comparison, the analyses of the carcasses of one normal, mature male rat, two normal males and two normal female rats, of the same litter and 75 days old. It also contains the analysis of 12 normal rat pups which were chloroformed a few hours after birth for this particular purpose.

It is to be observed that the results for calcium, magnesium, and phosphorus in the rats that were fed the synthetic diet, are approximately twice as much as the results for the corresponding elements in the carcasses of the two-year-old male and the 75-day-old male and female rats. This difference is accounted for in the main by the fact that the rats receiving the synthetic diet attained their maximum weights by the tenth week and declined in weight thereafter. Consequently the rats had lost a considerable proportion of their maximum weight at the conclusion of the

TABLE 3

Mineral matter contained in the carcasses of rats at their maximum live weights

	RATS CONFINED TO A SYNTHETIC DIET AND TO WHICH WAS ADDED SMALL AMOUNTS OF COMPOUNDS OF MN, CU AND ZN. DIGESTIVE TRACT EXCLUDED					MINERAL MATTER CONTAINED IN NORMAL RAT CARCASSES BEFORE DRYING. DIGESTIVE TRACT EXCLUDED		
Cage numbers	1	2	3	4	5	1 two-yr. old male rat	2 male rats. 75 days old Same litter as females	2 females. 75 days old. Same litter as males
Number of rats analyzed	3	4	4	4	3			
Treatments	Synthetic ration only	Ration + Mn	Ration + Cu	Ration + Zn	Ration + Mn, Cu, Zn	Steenbock ration	Steenbock ration	Steenbock ration
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Moisture H_2O	67.25	64.40	67.29	68.50	69.15	67.27	69.22	69.37
Copper, Cu	0.0003	0.0002	0.00046	0.00019	0.00052	0.00040	0.00050	0.00056
Iron, Fe	0.0040	0.0046	0.0042	0.0057	0.0067	0.0082	0.0084	0.0096
Manganese, Mn	0.00005	0.00008	0.00004	0.00003	0.00005	0.00013	0.00008	0.00011
Zinc, Zn	0.0025	0.0029	0.0018	0.0052	0.0052	0.0037	0.0039	0.0048
Calcium, Ca	1.387	1.5140	1.163	1.255	1.185	1.174	1.193	1.374
Magnesium, Mg	0.0557	0.0546	0.0679	0.0496	0.0439	0.0589	0.0526	0.0650
Phosphorus, P	0.7720	0.7988	0.6290	0.6930	0.7210	0.7410	0.8380	1.0260
Potassium, K	0.2116	0.1523	0.1543	0.1934	0.1824	0.3110	0.3335	0.4660
Sodium, Na	0.1530	0.1405	0.1547	0.1771	0.1807	0.2428	0.2436	0.2920

experiment and their dry carcasses contained a smaller proportion of meat and a correspondingly larger proportion of bone than the carcasses of the normal rats.

A point which appears to be of some significance is the fact that the largest percentages for calcium and phosphorus occur in the rats which received manganese in their diet (cage 2).

In table 3, the results have been calculated to the basis of maximum live weights of the rat carcasses. On this basis it is to be observed that carcasses of the normal rats contain considerably more iron than the rats confined to a synthetic diet which contained apparently an adequate amount of iron, which was added in the form of ferric citrate. The result for calcium in cage 2 is outstanding and apparently significant. The results for potassium in the experiments with the synthetic diets are con-

TABLE 4

Analyses by different authors of albino rat carcasses, moist basis, alimentary tract excluded

AUTHORS	AGE OF RATS IN DAYS	CALCIUM		MAGNESIUM		PHOSPHORUS		POTASSIUM	
		Males	Females	Males	Females	Males	Females	Males	Females
Buckner and Peter	84	0.73	0.71	0.024	0.036	0.51	0.49	0.25*	0.27*
Sherman and MacLeod	90	0.93	1.09	—	—	—	—	—	—
McHargue	75	1.19	1.37	0.053	0.065	0.84	1.03	0.33	0.47

* By an error in placing the decimal point, the percentages of K_2O given in the published paper are one-tenth of the actual findings. This error has been corrected in the table.

sistently lower than the results for potassium in the carcasses of the normal rats, and this relation also maintains with respect to sodium.

A point of unusual interest is the fact that the two female rats contained appreciably larger percentages of the various mineral elements than their brothers. This is very pronounced in the results for calcium, phosphorus and potassium and is significant.

PREVIOUS WORK PERTAINING TO THE SUBJECT. Since the albino rat is widely used for obtaining data from which the nutritional adequacy of foods is judged it might be expected that the mineral components of the carcass of normal albino rats had been well established. However, the fact remains that but few data have been published concerning even the major constituents of the carcasses of normal albino rats and as far as the writer is aware no data at all concerning the minor constituents to some of which it is quite conceivable to attribute important biological functions.

In 1922 Buckner and Peter (9) of this Station published results concern-

ing the CaO, MgO, P_2O_5 and K_2O in normal male and female albino rats varying in age from two to forty weeks. More recently (1925) Sherman and MacLeod (10) have published the results of a much larger number of analyses of the different sexes of normal albino rats varying in age from birth to 120 days. In table 4 are brought together the results thus far available concerning the major mineral constituents of the carcasses of normal albino rats as reported by three different authors.

In the foregoing table it will be observed that some wide variations exist between the results reported by the different authors concerning the percentages for the major mineral constituents of normal albino rat carcasses of near the same age. The 75-day-old male and female rat carcasses all of the same litter, analyzed by the writer contained considerably larger percentages of the various mineral constituents than is reported by either of the previous authors.

The two 75-day-old male rats had an average net weight (intestinal tract removed) of 75 grams and the two females an average net weight of 63 grams, while the 90-day-old males and females analyzed by Sherman and MacLeod weighed nearly three times as much. The 120-day-old male rats analyzed by Sherman and MacLeod had an average weight of only 35 more grams than the 90-day-old male rats. It is therefore apparent that the 90-day-old rats of Sherman and MacLeod had considerably more flesh in proportion to their age than the two normal male and female rats analyzed by the author which would have a tendency to give a lower result on the maximum net wet weight basis. However this line of reasoning does not apply to the normal mature male rat analyzed by the writer, the age of which was approximately 2 years and the net weight was 324 grams. The percentage of calcium in the carcass of this rat was only slightly less than the percentage of calcium in the two 75-day-old male rats analyzed.

The lot of stock rats from which the samples were taken was fed the Steenbock ration supplemented with table scraps, when available. Calcium carbonate is a constituent of the Steenbock (11) ration for stock rats which therefore supplied an adequate amount of calcium.

The author's results confirm and further emphasize to a greater degree the larger percentage of calcium in the females than in males of the same litter. This relation also maintains with respect to iron, phosphorus, potassium and sodium.

The author is of the opinion that appreciable losses may occur in the ashing of rat carcasses in open dishes without having previously destroyed the organic matter contained therein with nitric and sulfuric acids; however further work to prove this point is contemplated.

SUMMARY

Rats confined to a synthetic diet and under conditions that were the best that could be improvised at the time, gave results which the author interprets as indicating that compounds of manganese more definitely and possibly copper and zinc also have important biological functions in animal metabolism. Moreover it is assumed that the compounds of these elements as they occur in the natural state in green leaves, and seeds of mature plants and the vital organs of animals have a much more active biological potency than could be expected from feeding equal proportions of a crystalline salt of these metals in a synthetic diet.

BIBLIOGRAPHY

(1) McHargue, J. S. Journ. Agric. Res., 1925, xxx, 193.
(2) McHargue, J. S. This Journal, 1925, lxxii, 583.
(3) McHargue, J. S. Journ. Amer. Soc. Agron., 1925, xvii, 368.
(4) McCollum, E. V. et al. Journ. Biol. Chem., 1925, lxiv, 161.
(5) Scott, W. W. Standard Methods Chemical Analysis 1917, p. 165.
(6) Willard, H. H. and F. H. Greathouse. Journ. Amer. Chem. Soc., xxxix, 2366.
(7) Scott, W. W. Standard methods chemical analysis. 1917, p. 487.
(8) McCrudden. Journ. Biol. Chem., vii, 83.
(9) Buckner, G. D. and A. M. Peter. Journ. Biol. Chem., 1922, liv, 5.
(10) Sherman, H. C. and MacLeod. Journ. Biol. Chem., 1925, lxiv, 429.
(11) Steenbock, H. Science, November 30, 1923.

INTERMITTENT PULSE IN BLOOD PRESSURE INVESTIGATION

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In measuring arterial blood pressure by the auscultatory method the sounds at the elbow tend to become intermittent with a degree of compression which is near the systolic pressure. The groups of sounds heard and the silent intervals bear a relationship to the respirations which is important to ascertain, yet the subject seems to have received little attention. The variations are well seen in the graphic records as here produced from a study of the subject recently made by the writer in normal individuals.

METHOD. The respirations were recorded by means of a special stethograph, modified from the old Burdon-Sanderson form, and a recording tambour. The pulsations were registered by means of a sensitive Pachon oscillometer connected with the cuff and manometer system. The sounds were signalled by the observer using an ordinary spring key and electro-magnetic signal.

REMARKS. In a paper which recently appeared (1), the time of occurrence of the sound groups when intermittent was stated to be the expiratory phase of respiration, but, though it was said that graphic records had been taken, none were published. It had been previously held that the groups of sounds occurred during inspiration, the silent intervals corresponding with expiration (2).

The present investigation has been undertaken with a view to deciding the question, which is of much interest both physiologically and clinically. The work of Erlanger and Festerling (3), of Snyder (4) and others leaves the question undecided as to what the relationship between respiration and blood pressure is in man, and the general statement prevails that in some people the rise corresponds with inspiration and the fall with expiration, while in others the converse holds.

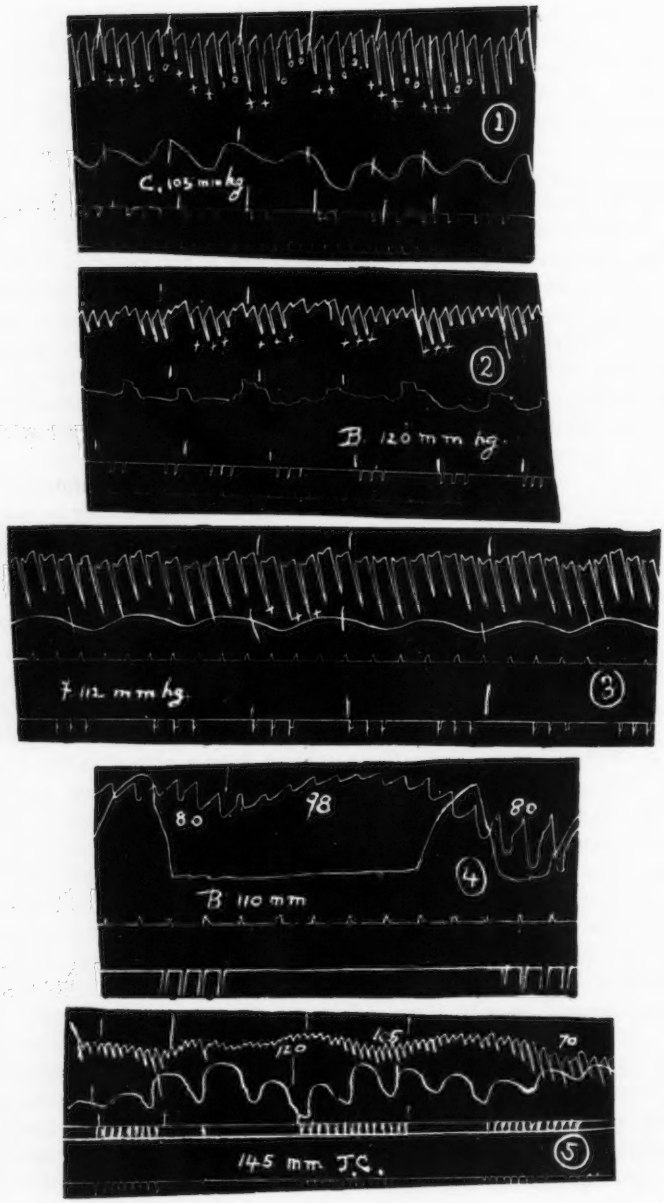
The rôle of brake has been ascribed (1) to the elastic traction of the lung in checking contractions of the ventricle. This connotes a relatively small output from the ventricle during inspiration, when the elastic pull is more marked than during expiration. The upholders of this view contend that in sclerosis of the lung, the effect of traction in inspiration being

considerable, a difference of 15 mm. Hg may occur in inspiratory and expiratory pressures, the *latter* being the higher. In artificial pneumothorax it is said, on the contrary the heart swells in inspiration and contracts without let or hindrance from lung traction, so that no such difference is seen. These pressure differences are also seen in normal individuals, even to the extent of 15 mm. Hg in some instances (1) and to them is ascribed the condition known as paradoxical pulse.

Thus then the basis of explanation of intermittence both in the normal and abnormal subject would be phasic differences in the elastic pull of the lung which acts against complete emptying of the ventricle in inspiration, while in expiration output is facilitated and blood forced through the compressed artery with the occurrence of sound in this phase. All this conflicts with the notion of the action of the respiratory pump in causing fluctuations of blood pressure where a rise coincides with the greater part of inspiration and a fall with the greater part of expiration, as in experimental curves from the dog, a notion which is believed by many physiologists to be near the truth for man.

Results. In over 50 per cent of normal individuals examined for the present contribution to the subject (25 in all) it was quite easy to note the occurrence of intermittence when the degree of compression was about equal to or slightly less than systolic pressure. Three or four sounds were distinctly heard, succeeded by 2, 3 or 4 pulsations in the oscillometer with no sound whatever. That is true intermittence; simple phasic variations of intensity and quality were noted in practically all subjects examined, but these are not specially considered in this paper. In some cases of intermission the sounds occurred in expiration and the time of onset varied from a point near the beginning of this phase to a point about the middle of the phase. This will be evident from a glance at the records (figs. 1, 2, 3, 4). Corresponding points in the tracings are frequently marked, so that there is not much difficulty in determining the relationship of events.

Sounds in the artery are supposed to indicate the passage of blood through a narrowed lumen (further reference will be made to this point later on) so that during their occurrence the pressure in the cuff is somewhat less than systolic pressure, whereas during the silent period the compression exceeds the systolic pressure. Fluctuations in the mercury level corresponding with these differences are not visible, though variations of the lever level of the oscillometer are obvious. The down stroke of this lever represents systole, and the lower levels correspond to higher pressures. The capsule of the Pachon instrument communicates directly with the cuff alone when actually recording, being shut off from the mercury manometer, pressure bag and general system. Communication with this main system is set up when it is essential to alter the pres-


1
C. 105 mm hg
2
B. 120 mm hg.
7 112 mm hg.
3
80
78
80
4
B 110 mm
120
45
70
145 mm J.C.
5

sure. In lowering the pressure from the exact point of complete obliteration of sounds to the point at which the sounds are continuous there is an average range of about 3 to 6 mm. Hg in those who show intermittence, and within this range the sounds are intermittent.

In a few cases intermittence of a different kind from this respiratory form was observed. In these the sound groups and intervals were much longer, each phase corresponding to three or four respirations, while the oscillometer curves showed more the form of Traube-Hering waves (fig. 5). In some others it was impossible to abolish the sounds altogether, even with very high compression. In these instances they were heard both proximally and distally to the cuff, in one case at 100 mm. Hg higher than the level of obliteration of the pulse at the wrist. The character of these sounds heard at very high pressures was, however, different from that of the ordinary sounds, that is, a clicking or tapping rather than a puffing or grating sound. It is this latter type of sound which indicates passage of blood through the vessel.

Discussion. With slight variations in the time of onset and cessation the sound groups are seen to correspond in the main with expiration, beginning soon after its inception. Sometimes there is a break in a silent interval by the appearance of a sound but in general the groups are regular. If the elastic pull of the lung, which varies with respiration, is to be considered the cause of intermittence the silent interval should be prolonged by holding the breath in inspiration and the sounds instead of forming a small group should be continuous when the breath is held in expiration. In the former case the elastic pull is sustained, in the latter it is relaxed over a longer period than usual. This test requires a good deal of care on the part of the observer and a little practice by the subjects so as to render the results reliable. Carried out with due precautions it yielded the following results: in prolongation of the position of normal inspiration the

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Fig. 1. Curves: top line, oscillometer record; second, respiration (upstroke inspiration); signalling of sounds; time in seconds. Pulsations marked $\pm$ correspond to sounds signalled; those marked *o* give no sounds. Downstroke of oscillometer systolic. Low pressure, 105 mm. Hg.

Fig. 2. Order of curves as in previous figure. Nervous individual with jerky respiration. Medium pressure 120 mm. Hg.

Fig. 3. Steady individual in whom intermittence of very regular type prevailed over long periods within pressure difference of 6 mm. Hg. (108 mm.–114 mm). Very slight variations in time of onset of sound groups. Bottom line—signal.

Fig. 4. Showing the suppression of sounds in case of intermittence when breath is held in normal expiratory phase. The slowing of the pulse coinciding with sound groups is well seen.

Fig. 5. Long groups of sounds alternating with long silent intervals covering a period of 2 or 3 respirations. Third record—signal. Obviously vasomotor effect. Slowing of the pulsations coincides with sounds.

silent interval is always prolonged and in some instances no sound may be heard for a period of 15 to 20 seconds. When the breath is held in the normal expiratory position the sound group either ceases at the usual point or continues for one or two beats longer, but there is always a succeeding silent period whether short or long. A rise of 1 to 2 mm. Hg such as might easily occur from straining or muscular effort may cause a continuance of the sounds, and this sometimes makes the observation difficult, but with care to avoid this one meets with many cases where one has no doubt of the suppression of the sound in expiration and one can repeat the observation over and over again (fig. 4). Though in a few instances the sounds recurred, the breath being still held in expiration, at about the usual period of the intermittence,—that is the intermittence was not interfered with by the prolonged expiratory state,—the more usual result was total suppression for periods of 8 to 12 seconds; then a rise of blood pressure occurred with accumulating CO_2 and the sounds returned. A forced expiration supervening on a normal expiration, at which the breath has been held for a few seconds previously and during which there is silence, may cause the sounds to recur, but sometimes it does not. Slow continuance of the expiratory act to expel supplemental air causes the sounds to continue.

What is the significance of these observations? Obviously the results obtained with prolongation of normal expiration are opposed to the view that blood pressure rises with expiration *because* of diminution of lung traction as a hindrance to emptying of the ventricle. If this were so the pressure should at least remain high enough to cause the sounds to continue when the slackness of lung pull is sustained. The effect of maintaining inspiration, viz., the prolongation of the silent period is not opposed to the view mentioned, but in the light of the expiratory result it cannot be said to go very far in support of it.

What then is the cause of intermittence? And what is the cause of the discrepancy between the curves seen and the classical blood pressure curves of experiment in their relations to respirations? The method of signalling the sounds here adopted may be considered reliable. The signals correspond pretty well with those beats on the oscillograph record where sounds would be expected and accompany the rising pressure curves on this record. The sounds signalled by two observers only are given and these had thorough practice in the method.

The return of an increased amount of blood to the left auricle when the lung volume is decreased in expiration should not produce an instantaneous effect on the brachial pulse, though the explanation usually given of the continued rise of pressure in experimental curves during the early phase of expiration connotes such an immediate effect on the left side of the heart. With a certain short interval allowed for the blood which is

squeezed from the lung in expiration to affect the blood pressure this increased flow would fit in as a factor in the causation of an expiratory rise corresponding to the time in our present records. That this is a factor in the causation of periodic rise of pressure is a view which is favoured by the observations made in expiratory positions. When a dog is being bled and is breathing naturally it is easy to note that when the stream is getting feeble and the pulmonary ventilation increases the flow becomes intermittent. There are gushes of blood in expiration and few drops during inspiration. The flow is best marked toward the end of the former phase. So even in the dog when the type of respiration is changed we find a change in the normal blood pressure relations to respiration, providing a parallel for what has been found in the present investigation to be most common in man, viz., a rise of pressure in expiration and a fall in inspiration.

That the ordinary respiratory variations of intrathoracic pressure should influence the filling and emptying of the thick walled ventricle to the extent connoted by the view put forward above is altogether unexpected. In quiet respiration the difference in intrathoracic pressure does not amount to more than a few millimeters Hg. The ventricular wall is quite indifferent to much greater changes of pressure within, sending out the same quantity of blood when intraventricular pressure is high as when it is low, accordingly a change of a few millimeters Hg in extraventricular pressure should not cause such a difference in the force of systole as to diminish output.

A reflex or central modification of activity in cardiac nervous mechanism has been invoked as a cause of the respiratory changes of blood pressure. The results of cutting the vagi show that such a factor must be taken into account. Variations of vasomotor activity are certainly responsible for those longer fluctuations of pressure which are seen in figure 5.

Relationship to rate of heart beat. Howell (5) and others draw attention to variations of heart rate as a cause of variations of pressure. In the dog it is common to find a faster rate in inspiration and to this is ascribed in some degree the higher blood pressure in this phase. This difference in rate is occasionally found in man. Two individuals in the present series exhibited slowing of the beat in expiration, but this slowing marked the rise of pressure, not the fall, as judged by the occurrence of sounds and the appearance of the curves of the oscillometer (figs. 4, 5). These slow beats generally show a greater amplitude than those of the fast rate, and the mere fact of slowing is said to account for this greater size without considering increased force. Judging by the loudness of the corresponding sounds and the accompanying rise of pressure one cannot help coming to the conclusion that the force of these slow beats is at least sometimes increased. These sounds are long and loud and undoubtedly

mean a comparatively large volume of blood being forced through the cuff with each. The respiratory rise of blood pressure, whether expiratory or inspiratory, means increased output in a given time which connotes increased venous return during that period; and enhanced efficiency of the cardiac mechanism might well be exhibited in a reduction of rate and augmentation of force.

To fluctuations of activity in the cardio-inhibitory centre are attributed changes of rate which in turn have been invoked as a cause of changes in pressure. The cardio-inhibitory centre may be influenced in two ways, viz., reflexly through the vagus and by overflow from the respiratory centre. This change of rate to whatever cause it may be assigned is too infrequent to account for constant variations of blood pressure which accompany respiration and in ordinary breathing must play a very small part, if any.

CONCLUSIONS

Changes in blood pressure accompanying respiration in man are more commonly of the nature of a rise in expiration and a fall in inspiration than of a converse type found in the dog.

Intermittence of the sounds at the elbow is common in investigating blood pressure by the auscultatory method. This phenomenon is apparent at a pressure in the cuff which is a little below systolic pressure.

The sound groups occur during expiration and the silent interval during inspiration.

Fluctuations of pressure are due to variations in the return of blood from the lungs which is greater in expiration, but the moment of onset of rise is not constant.

A slowing of the pulse may accompany the rise of pressure. There is probably a nervous factor involved in the respiratory changes of blood pressure, working through the vasomotor centre rather than the cardio-inhibitor. Variations of the elastic pull of the lung in respiration do not affect the output from the left ventricle.

BIBLIOGRAPHY

(1) Rist et Walser: Journ. Med. Bordeaux, 1925, xix, 842.
(2) Constantin et Soula: Quoted by Rist and Walser.
(3) Erlanger and Festerling: Journ. Exper. Med., xv, 370.
(4) Snyder: This Journal, 1915, xxxvi, 430.
(5) Howell: Text book of physiology, 1924.

THE DURATION OF THE SYSTOLE OF THE LEFT VENTRICLE OF MAN

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At the time this study was begun, in 1917, the medical journals contained many articles dealing with cardio-vascular disturbances exhibited by soldiers. The general uncertainty as to the etiology of these conditions was shown by the numerous and varied names given to them by even the most careful observers. It occurred to us that if there could be found a test of the efficiency of the cardiac muscle, as such, it might clear up a part of the difficulties of diagnosis, and possibly, as well, throw light on the prognosis of cases which might otherwise be obscure, by distinguishing between the results of a degenerated heart muscle, and the effects of imperfect valve action. One recalls Sewall's (1) statement: "Thus an organic heart lesion, however obtrusive its physical signs, may no wise impair the efficiency of the body in the execution of its daily tasks. Functionally therefore, it is as if the engine of the circulation were perfectly healthy."

In seeking a test of the condition of the heart muscle, we recalled the well-known fact that the muscles of animals, when fatigued, or degenerated, not only make feebler contractions, but have longer latent periods, and require more time to complete their contractions. Storey (2), working in this laboratory, found this to be true of fatigued human striated muscle. It seemed not unlikely that the human heart muscle would behave in a similar manner, and we decided to make a careful study of the duration of the period of contraction of the muscle of the left ventricle of the normal human heart, in the hope that the duration of the systole might serve as a gauge of the condition of the heart muscle and its ability to do work. We (3) have briefly reported the results which we obtained in this study, from time to time, but this is the first extensive report.

We wish to say at once that we have found that the duration of the systole is influenced so largely by the quantity of venous blood supplied to the heart, that this factor may disguise the effect produced by the condition of the heart muscle. Evidently the use of the length of the systole as a gauge of the condition of the heart muscle must depend on future work which will take into consideration the quantity of blood to be pumped.

The most accurate method of measuring the time occupied by the systole of the left ventricle is probably that developed by Wiggers and Dean (4), in which they recorded optically the interval between the first and second heart sounds. Because of the highly technical character of the apparatus involved, we decided to use the simpler method of estimating the contraction time of the ventricular muscle by measuring the systolic portion of the carotid sphygmogram, thinking that, if it proved satisfactory, it might be more readily employed. Bowen (5) used this method very successfully in a series of experiments on the pulse and the systole of the heart, in this laboratory, in 1903 and 1904.

We measured the time between the beginning of the rise of the primary wave and the bottom of the dicrotic notch (*b–d*, fig. 1), and called this interval the systolic time. Of course we were aware that the left ventricle

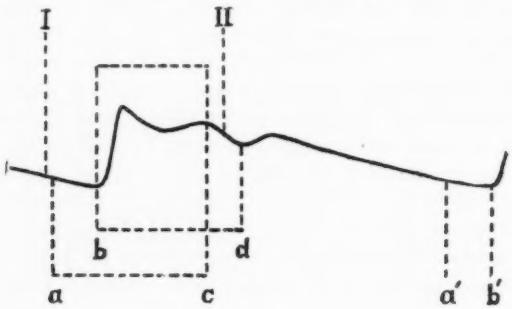

Fig. 1. A spygmogram of the carotid pulse. *a—c*, true systole; *b—c*, period of discharge; *b—d*, time measured as systole; *c—a′*, true diastole; *d—b′*, time measured as diastole; *I*, *II*, 1st and 2nd heart sounds in relation to time intervals of carotid pulse.

begins to contract before the pressure which it develops becomes great enough to overcome the diastolic pressure in the aorta and to open the semilunar valve. Even allowing for transmission time, the primary upstroke of the carotid pulse starts later than the beginning of the actual systole of the ventricle, by an interval *a–b*, figure 1, and the relaxation of the ventricle probably begins with the fall of the descending limb of the dicrotic notch, or earlier by the interval *c–d*, figure 1, than the time we measured.

It seemed to us, however, safe to assume that the interval *b–d*, figure 1, although beginning later and ending later than the true systole, would last approximately the same time. Marey (6) regarded the point of origin of the dicrotic wave as marking the division between the systolic and diastolic portions of the pulse curve, and Hürthle (7) said,—"allein die

folgende Betrachtung zeight, dass die Strecke vom Beginn des Pulses bis zum Auftreten der dicrotische Welle ziemlich genau die Dauer der Kammersystole darstellt, obwohl sie sich nicht genau mit dieser Phase der Herzrevolution deckt."

According to Robinson and Draper (8), "It should be emphasized that in measuring the lengths of systole and diastole on a sphygmogram in which the lowest point of the dicrotic notch of the carotid sphygmogram is used as the point where systole ends, the presphygmic period must be added to the measurement of the systole and subtracted from the measurement of the diastole to give the true length of each of these phases." They seem to neglect the fact that the interval between the end of the systole and the bottom of the notch, *c–d*, is almost as long as the presphygmic period and so nearly compensates for it.

After we had determined the duration of the systole in a sufficient number of cases, we tried to compare our results with those of others who had worked in this field. Unfortunately we found this impossible in many cases. Our difficulty was that different observers gave different boundaries for the systole—a matter of definition; very few observers had stated the position and sex of the subjects; some had tested but few subjects; and many gave only the maximal and minimal duration of the systole—figures which were of little value for our comparison, as there may be an enormous difference in the length of the separate systoles. The results of the most important of these observations are summarized by Wiggers (9) in the second edition of his *Circulation in Health and Disease*, pages 102 and 206, respectively. References are given by Tigerstedt (10), Bazett (11) and Katz (12).

A few writers (11), (13), (14), (15), have developed formulae permitting the calculation of the duration of the systoles at different pulse rates, but we have found none of them satisfactory.

The estimation of the length of the systole based on the idea that the heart sounds bound the systolic period, probably gives a time which is a little long. Apparently the same mechanical conditions determine the closure of both the auriculo-ventricular and the aortic valves, viz., the elastic recoil of the wall of the ventricle and the sudden fall of pressure in the auricle, in the case of the former, and the elastic recoil of the aorta and the sudden fall of pressure in the ventricle, in the latter case. The first sound probably occurs a very little before the beginning of the contraction, and the second, a little after the beginning of the relaxation of the ventricle. (See *I* and *II*, fig. 1.)

Our results correspond most closely with those of Wiggers and Clough (16), who used the heart-sound method, and the sitting position; and of Eyster (17), who employed the *C* wave of the venous pulse and the second sound, with the subject in the recumbent position; but our estimates are

roughly 0.010 to 0.020 second shorter than theirs, depending on the pulse rate. Indeed, almost all observers have found the duration of the systole to be longer than we have estimated. This fact made it probable that our method failed to give the exact length of the systole. If there was an error, it undoubtedly lay in our assumption that the time occupied by the fall of the descending limb of the dicrotic notch compensated for the isometric period. After we had developed our standards, we attempted to measure the time required for the fall of the descending limb of the notch. This cannot be determined with great accuracy, because of the difficulty of deciding the exact instant at which the fall begins; nevertheless, an average obtained from the measurement of fifteen consecutive cycles gives a fairly accurate estimate for the curve of the individual, and an average obtained from 25 subjects is fairly accurate for the group. The average of a considerable number of figures is needed, because the time occupied by the fall of the descending limb of the notch is not constant; it is subject to a considerable number of influences; for example, it may vary with the respirations, the variations being very evident when the height of the sphygmograms undergoes extensive changes. In general, when the notch is deep, the fall is more rapid than when the notch is shallow, but the time required for the fall is, nevertheless, longer; no constant relation was found.

We obtained the following figures for the average time required for the fall of the descending limb of the dicrotic notch: Standing position, 0.035 second; sitting, 0.038 second; recumbent, 0.042 second. The differences are probably due to the way the heart and aorta react when smaller and larger quantities of blood are being pumped by the heart, under varying conditions of arterial pressure.

The isometric period has been found to be subject to considerable variations, and the figures which have been given by different observers differ considerably (see Robinson and Draper (8)). Most workers failed to state the position of the subject, and yet the position influences the amount of blood which the heart has to pump, and would probably affect the isometric period. The following estimates of Wiggers and Clough (16) and of Robinson and Draper (8) show that this is the case, and it is not unlikely that this phase would be influenced by the respirations. The following tabulation of averages suggests that the standards which we have determined, give too short systoles for the sitting and recumbent positions.

Isometric period, sitting	0.050 second	(Wiggers, Clough)
Fall of descending limb of notch	0.038 second	(Lombard, Cope)
Systoles too short, by	0.012 second	
Isometric period, recumbent	0.076 second	(Robinson and Draper)
Fall of descending limb of notch	0.042 second	(Lombard, Cope)
Systoles too short, by	0.034 second	

As the difference is less for sitting than lying down, it is probable that it would be still less for standing than sitting. It is evidently too soon to speculate further until we have more evidence with respect to the isometric period, which one of us is studying at present.

Although a knowledge of the length of the systole of the human heart is of some value, the actual length of the ejection period is also of importance. The period of ejection corresponds to the interval shown on the carotid pulse curve between the beginning of the rise of the primary wave and the point at which the dicrotic notch begins to form, *b–c*, figure 1. This interval is shorter than the duration of the systole, as we have measured it, *b–d*, figure 1, by the time required for the fall of the descending limb of the dicrotic notch, *c–d*, figure 1, which averages for the different positions of the body, viz., 0.035 second in the standing, 0.038 second in the sitting, and 0.042 second in the recumbent position, in the case of men having ordinary pulse rates after resting. We do not know what would happen at quicker rates. As will be shown later, at higher pulse rates the systoles may be shorter than the standards which we have developed, and the time for the fall of the descending limb of the dicrotic notch may be longer.

That there may be no misunderstanding, we repeat that we have measured on the carotid pulse curve the time from the beginning of the rise of the primary wave to the end of the fall of the descending limb of the dicrotic notch—the P–D times,—and assumed that it closely approximated the duration of the systole.

The duration of the systoles as stated in this article, is the average duration of at least 15 consecutive cycles. A sharp distinction must be made between the length of the separate systoles and diastoles of suceeding cycles, and the average length of the systoles and disastoles. The length of succeeding cycles may vary much more than is generally recognized. Many observers have noted that the heart quickens in inspiration, and Lombard and Pillsbury (18) pointed out that the pulse may have a vasomotor rhythm also. As a result of these influences, the rate of the heart undergoes a compound rhythm, and may alter considerably within short intervals of time. If one estimates the change of heart rate per minute from the length of each of the succeeding cycles, it will be found that the pulse may change its rate 20 beats in the course of a minute. Not only do succeeding systoles and diastoles vary in length, but they do not always vary in the same direction. It is for this reason that we have found that the average of 15 successive cycles must be employed to determine the average length of the systoles of a given subject at a given time. We hope to publish another paper dealing with the variations of the systoles, diastoles and cycles.

As will be explained later, we have developed a standard length of

systole, based on cycle length, for each of the positions of the subject,—standing, sitting and recumbent. We have found that none of the presumably normal subjects examined had systoles the average duration of which differed from the averages which we regard as standard, more than 0.025 second, plus or minus. This relatively large variation, which will be discussed later, is, without doubt, in part caused by errors of method, but is probably due much more to variations in the amount of venous blood returning to the heart.

The results which are the basis of this paper were obtained by 252 tests made on 176 men standing; 94 tests on 91 men sitting; and 66 tests on 64 men in the recumbent position. Inasmuch as at least 15 and in a number of cases 30 or more cycles were measured in each test to obtain the average length of the systole, the total number of cycles in which systoles and diastoles were estimated, and which form the basis of this paper, was more than 7000. This does not include our studies on the systoles of women, the effect of exercise, and the pathological cases which we have examined, which enter into this paper only incidentally.

In conclusion, we started this research in the hope of finding a test of the ability of the heart muscle to do its work, and at present can only say that the duration of the systole is markedly altered by the pulse rate; by the position of the body (because of the effect of gravity on the amount of blood supplied to the heart); and by sex. The time of year, age (15 to 65 years), height, weight, ordinary blood pressure, and smoking, failed to have any noticeable effect. Finally, the systolic time of ordinary normal men does not differ more than 0.025 second plus or minus, from the average length of the systoles of other normal individuals in the position in question and having the same pulse rate.

Apparatus and method. The method employed to record the duration of the systole is, of course, of great importance, and the different methods used by different observers probably account for the fact that the figures given by careful workers differ so much, not only from ours, but from each others. In all our experiments we recorded the respiration, the carotid pulse, and the time, on an endless band of blackened paper 12 cm. wide and 170 cm. long, moving at the rate of 50 mm. per second, so that a long continuous record could be taken. The time was written by an electrically driven tuning-fork having a rate of 50, or sometimes 100, double vibrations per second.

We obtained the pulsations of the carotid by means of an *open receiving tambour*, which consisted of a metal box 5 cm. in diameter and 12 mm. deep, which was fastened to the neck by a U-shaped spring having at one end a ball which fitted into a socket in the middle of the box, and at the other end a button of vulcanite 3 cm. in diameter, which rested against the side of the neck opposite the tambour. The arrangement permitted the tam-

bour to adjust itself to the form of the neck, and give a constant pressure throughout the experiment.

The recording tambour (see fig. 2) was of a form which we have used for many years in this laboratory with very satisfactory results. It consisted of a brass cup 10 mm. in diameter and 13 mm. deep. The lever, which was 10 cm. long and which magnified 50 times, was a very thin strip of bamboo with a delicate paper point. The membrane was of the thinnest rubber dam obtainable, and had a tension which gave pulse tracings about 5 mm. high. The tambours had a vibration frequency of 12–13 per second.

The pneumograph. The respirations were recorded with the aid of a pneumograph of the old Marey type consisting of a rubber tube 2 cm. in diameter and 8 cm. long, kept open by a coiled spiral spring within.

The rubber tubes connected with the tambours were long enough to allow the records to be taken from the patient when standing, sitting, or when lying on a bed beside the table on which the kymograph was placed.

The bed was a broad board supported by an axis which allowed the subject to be tilted to any desired angle, head up or down, while the record was being taken.

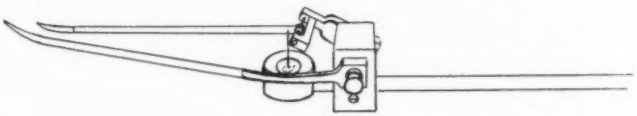

Fig. 2. Recording tambour

Arrangement of the apparatus. The recording tambours were mounted close together on a stand which could be rotated as a whole, to permit changing the friction of the writing-points on the blackened paper while the record was being taken. This was very important, for any excess of friction modified the record. Of course each record was preceded by arcs written by the levers, and not only axis lines were written for each tambour, but a base line was written a short distance below the tuning-fork curve.

Routine of making tests. The subject was given a number, and his history was entered on a filing card which held spaces for the date, his number, name, age, height, weight, present and past occupation, general condition, past illnesses, use of alcohol, use of tobacco, athletic sports, and systolic and diastolic arterial pressures. While the history was being taken, the subject sat quietly for five or more minutes, to permit recovery from any previous exercise. The sytolic and diastolic pressures were then taken by the Korotkow method, and always by the same man. In some of the later cases the systolic and diastolic pressures were taken in the sitting, standing, and recumbent postures just before the test for the length of the systole. During the test of the length of the systole, in the standing

position at rest, the subject stood beside the apparatus table, and his carotid pulse and respiration were recorded, the record being watched to be sure that at least 15 successive reliable cycles were written. In certain cases the record was taken for a full minute. After the standing test, the subject sat down for a few minutes, and a record was taken in this position. He then reclined on the horizontal bed, and after five minutes, another record was obtained.

Peculiarities of the sphygmogram due to the adjustment of the receiving tambour on the neck. It is important that the receiving tambour should be adjusted directly over the course of the carotid artery. If this is not done, an inverted pulse tracing may be observed, see figure 3. Mackenzie (19) states, "If we place one receiver over the carotid and one alongside it, and have the movements properly registered, the one tracing will be found to be the exact reverse of the other. If the artery expanded during the ventricular systole, it would naturally thrust out all the tissues surrounding it, and the tracing from the side would then be an exact duplicate of the one taken from the front of the artery. The movement, then, of the beating carotid is one of displacement of the whole vessel, not a dilation and contraction of the vessel." Keyt (20) has the same view, and says this was first noted by Marey in 1874. In our case, however, the tambour appeared to be rocked; the tambour was too far to one side, so that one edge of the rim rested over the artery, and when it pulsated the tambour was raised and lowered, causing the lever to move in the direction opposite to what is ordinarily seen when the tambour is properly placed directly over the artery and the skin as a whole pulsates beneath it.

It occasionally happens, even when the tambour appears to be properly applied, that the primary rise of the pulse tracing is preceded by a slight depression of the curve. See figure 4, which gives an exaggerated example. The dip of the curve is probably associated with the isometric period of ventricular contraction. Frank (21) has referred to such a negative impression, and Wiggers (22) has noticed it in optical arterial tracings, and believes it to coincide with the beginning of the intraventricular pressure rise and consequently to indicate the beginning of systole. In our tracings it was usually too indefinite to be utilized, but in test 249, the case that is illustrated in figure 4, it was measured in 25 cycles, and averaged 0.051 second, with variations, apparently respiratory, of 0.042 to 0.066 second, figures which compare well with those given by Wiggers and Clough (16) for the isometric period of man—0.040 to 0.060 second.

The auricular wave. When the subject is lying down, a slight swell of the curve is not infrequently seen to precede the primary upstroke, see figure 5. This appears to be due to a contraction of the left auricle. Several writers have noted the appearance of auricular waves in sphygmograms (22), (23) (24). In the recumbent position, the venous blood not

being held back by gravity, as in the standing and sitting positions, returns more rapidly to the right heart, which in turn drives more blood per beat

Fig. 3. Inverted sphygmogram due to maladjustment of receiving tambour.

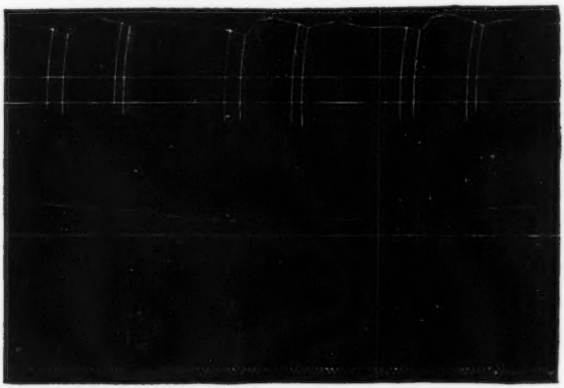

Fig. 4. Sphygmogram showing depression preceding primary rise.

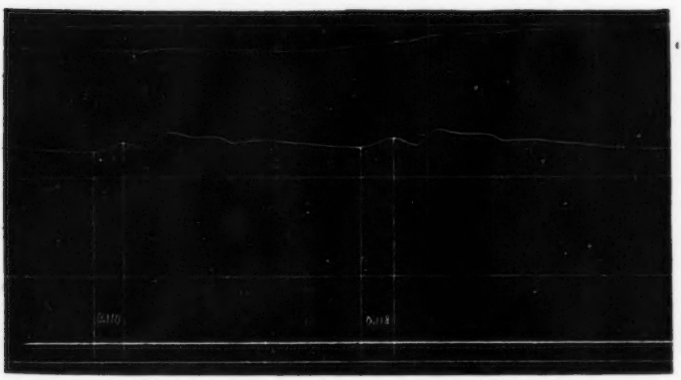

Fig. 5. Sphygmogram showing auricular wave.

through the lungs to the left auricle, and the sudden distention of the left ventricle caused by the beat of the left auricle gives the root of the aorta a shove, which causes a pressure wave to be transmitted to the carotid.

It suggests itself that this is merely a contamination of the carotid curve by the ordinary auricular wave in the veins of the neck. None of the other characteristics of the venous pulse, however, appear in the record, and the pressure of the tambour, as we apply it to the neck with the aid of a spring would close off the superficial veins. Although indicated on many curves, it is rarely clearly defined. We have such a record in which the duration of the auricular wave varies in height and length with the respirations, and can be quite accurately measured. If our explanation be correct, the wave could give an indication of the action of the left auricle of man, just as the auricular wave of the venous pulse gives evidence of the action of the right auricle.

The reading of the curves. Attention has just been called to the fact that the primary wave is sometimes preceded by a slight depression of the curve, which makes it difficult to decide just where the primary wave begins to rise. Moreover, not infrequently the dicrotic notch seems to be prolonged, the ascending limb not rising promptly, so that one is in doubt as to just what point is to be regarded as the bottom of the notch. In all cases one has to study a number of pulse beats before beginning to draw perpendiculars to the base line. *According to our method of measurement, the length of the systole is the time between the point where the tracing first begins, not simply to curve, but to bend suddenly upward at the foot of the primary rise of the sphygmogram, and the point where the descending limb of the dicrotic notch first ceases to fall.*

At least 15 succeeding cycles were chosen to be read and then numbered. A heavy steel straight-edge, 2 feet long, was placed accurately on the base line beneath the tuning-fork curve, and, a celluloid triangle being used, verticals marking the beginning of the primary rise, and the end of the fall of the descending limb of the dicrotic notch of each of the cycles, were drawn with a teasing needle through the time line. This was done under a large reading glass, so supported on a standard that the glass could easily be turned horizontally through a wide angle, and brought in succession over several of the pulse tracings. In a few cases, the pulse tracing was displaced so much from the horizontal by respiratory movements, that it was necessary to draw arcs to the axis line of the lever before the verticals could be drawn.

The tuning-fork vibrations were then counted for each successive systole and diastole. With a little practice one can mark one vibration, see four, and mark the fifth with considerable rapidity. He has then only to count the fives and the extra vibrations, and to estimate by the eye the tenths of a vibration where fractions occur at the boundaries. After the verticals which mark the beginning and end of the systoles and diastoles have been drawn, the errors of reading ought not to be more than 0.002 second, and these disappear when the measurements of 15 cycles are

averaged. The errors which depend on the drawing of the verticals are, without doubt, much greater. In the interest of uniformity, we adopted the plan of having all curves read by the same man, and doubtful estimates checked by the other. In spite of this it was of course impossible to establish with absolute certainly the beginning and end of the systole. When any great doubt existed, the curve was discarded.

What should be considered the pulse cycle? The question naturally arises whether one should consider a pulse cycle to begin with the systole or the diastole. The natural view seems to be to include in a pulse cycle a systole and a following diastole, rather than to add a systole to a preceding diastole. We have adopted the former method, although the results would be the same whichever method of recording the cycle was adopted, because the length of the systoles and diastoles of every subject was determined as an average of the figures obtained from the measurement of 15 consecutive cycles.

How many cycles should be read? This is a question of practical importance, because of the time and labor involved in reading, tabulating, and averaging results. The reading and averaging of 5 cycles gives little more information than a single cycle. Ten cycles may not give a reliable average, but 15 cycles, because the variations due to respiratory and vasomotor influences are averaged out fairly well, ordinarily give as accurate results as 30 or more.

Method of recording the results. After the length of the series of systoles and diastoles of 15 consecutive cycles had been estimated, the average length of the systoles, diastoles and cycles was computed with the aid of an adding machine. The approximate average pulse rate was obtained by reference to a table. The record sheet of the experiment contained places for name and number of subject, date and time of test, systolic and diastolic arterial pressures, and duration of the systoles, diastoles, cycles and pulse rates of each cycle (these being arranged in a series numbered to correspond to the numbers which had been placed on the curves as the cycles were measured), the limits of the variations of the systoles and diastoles, and finally the amount that the average systole differed plus or minus from the standard.

Errors in reading were often suggested by the extent of the variations in the length of the systoles. Errors attracted the attention still more, when the length of the diastoles and systoles were plotted one above the other as curves, these periods being the ordinates and the cycle lengths the abscissae. Any marked deviation of a given measurement from those immediately preceding and following stood out as exceptional. Inasmuch as every curve carried the number of the subject, and each cycle which was read was numbered, only a few minutes was required to find and remeasure a doubtful cycle.

Still another type of record was kept for convenience of ascertaining the effect of special influences on the length of the systoles. These were cards (see fig. 6), which could be readily arranged according to the influence to be studied, and saved a great deal of time.

DURATION OF THE SYSTOLES OF MEN IN THE STANDING POSITION IN RELATION TO LENGTH OF CYCLES. In our early work (3) we estimated the length of systoles with respect to pulse rates, and found that the resulting curve was a parabola, whereas when the systoles are plotted against cycle lengths, the resulting curve is a straight line. The relation of the values of the two curves may be seen in table 3 in which equal decrements of cycle length are shown to correspond to progressively larger increases in pulse rate. Either standard might be used for determining the relations of systole length to the heart's revolution, but in practice the use of the cycle length is more convenient.

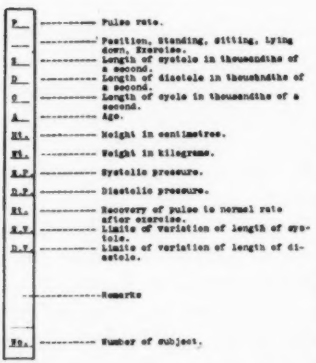

Fig. 6. Card used in studying effect of possible influences.

To establish a standard for the normal length of systoles in all ordinary cycle lengths of men standing at rest, we proceeded as follows: With cycle lengths as abscissae and systole lengths as ordinates, we plotted the average duration of the systoles of the normal men whom we had examined, and obtained a broken curve, which declined approximately as a straight line, systoles shortening as cycle lengths decreased. To obtain the height and pitch of this line more accurately, we arranged the figures giving the duration of the cycle in the descending order of length with their respective systole lengths opposite them, and found the average cycle and systole lengths. This gave us two values (see table 1) which we used to establish the mid-point of the standard curve. In like manner we found the average cycle and systole lengths of those cycles which were respectively longer and shorter than the average of the whole, by which we obtained two other points. These points (dots surrounded by circles in fig. 7), of course lay in a straight line. The height of this line was determined by the position of the mid-point, and its pitch was determined by the positions of the upper and lower points. This standard curve was based on the average of 15 cycles measured on each of 252 tests made on 176 men; in all, 3780 cycles. The longest average cycle length of any individual was 1.144 second (pulse rate 52.5), and the shortest, 0.423 second (pulse rate 142). The high pulse rates were not a result of exercise, but of mental excitement.

TABLE 1

The table gives the averages, in seconds, which were used in plotting the curves of the duration of the systoles of men, shown in figures 7 and 8.

	CYCLES	SYSTOLES	DIASTOLES	PULSE
252 tests on 176 men when standing after resting				
Average of first half	0.8314	0.2490	0.5824	72.17
Average of total tests	0.7324	0.2341	0.4983	81.92
Average of second half	0.6333	0.2192	0.4141	94.74
94 tests on 91 men in the sitting position				
Average of first half	0.9196	0.2831	0.6365	65.35
Average of total tests	0.8156	0.2677	0.5479	73.57
Average of second half	0.7117	0.2523	0.4594	84.30
66 tests on 64 men recumbent				
Average of first half	1.0466	0.3110	0.7356	57.33
Average of total tests	0.9453	0.3003	0.6450	63.47
Average of second half	0.8441	0.2897	0.5544	71.18

TABLE 2

The table gives the averages, in seconds, which were used in plotting the systoles of women.

	CYCLES	SYSTOLES	DIASTOLES	PULSE
72 tests on 68 women, standing after resting				
Average of first half	0.7604	0.2570	0.5034	78.90
Average of total tests	0.6952	0.2467	0.4485	86.31
Average of second half	0.6301	0.2364	0.3937	95.22
58 tests on 58 women, sitting				
Average of first half	0.8160	0.2785	0.5375	73.53
Average of total tests	0.7477	0.2693	0.4784	80.24
Average of second half	0.6794	0.2601	0.4193	88.31
58 tests on 58 women, recumbent				
Average of first half	0.8845	0.3053	0.5792	67.83
Average of total tests	0.8057	0.3003	0.5054	74.47
Average of second half	0.7268	0.2952	0.4416	82.55

To test the matter farther, we divided all the data, arranged as before, into groups of cycles differing not more than 0.050 second, found the

average cycle and systole lengths of each group, and plotted them on the curve. The agreement was as close as could be expected from the number of tests. In only 4 of the 15 groups (19 out of 252 tests), was the deviation more than 0.004 second. The result of the comparison made it appear that within cycle lengths 1.000 second to 0.500 second (pulse rates 60 to 120) at least, average systole lengths shorten at a regular rate as cycle lengths decrease, the curve of the decrease in the duration of the systoles,

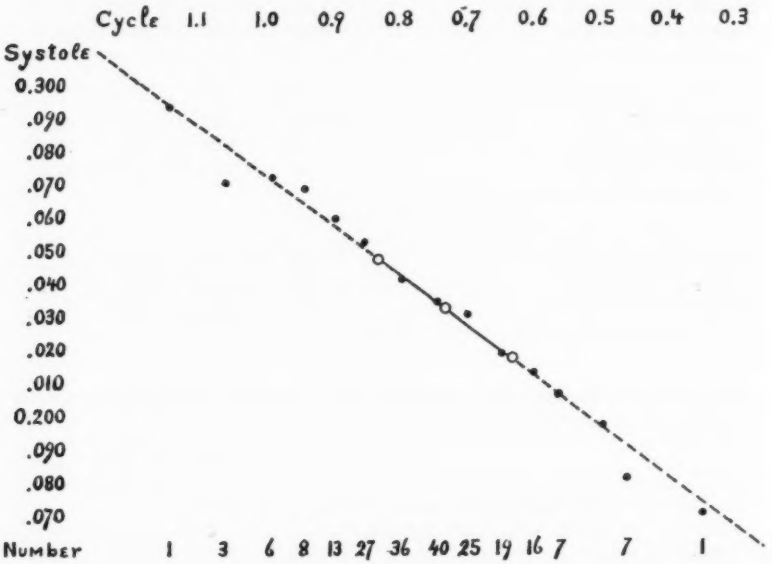

Fig. 7. Standard curve showing rate of decrease of length of systoles as cycles shorten, in the case of normal men standing after resting. Figures at top of curve give cycle lengths in thousandths of a second; ordinates, duration of average systoles in thousandths of a second; dots surrounded by circles give location of the three points by which the standard curve was constructed; dots placed along the curve represent average systole lengths of successive groups of cycle lengths differing not more than 0.050 second; figures at the bottom show the number of cases in the successive groups just mentioned.

plotted with cycle lengths as abscissae, being a straight line. Whether the same rate of change persists for longer and shorter cycle lengths is uncertain.

Effect of position. Quite early in our work we found that students, when measuring the length of systoles, during the laboratory course, all found times which were much longer than those which we were obtaining

from men who were standing after resting. In seeking an explanation, we recognized that the students were recording the carotid pulse in the same way we did, but that the subjects were sitting while the tests were being made. We decided to see if the position of the subject made any difference, and found that it was invariably the case that men examined in the sitting position gave longer systoles than the same men when standing. Of course we tried the effect of the recumbent posture, and ascertained that the systoles were even longer in this position. From this time on, all subjects were examined in all three positions. In addition, we tested

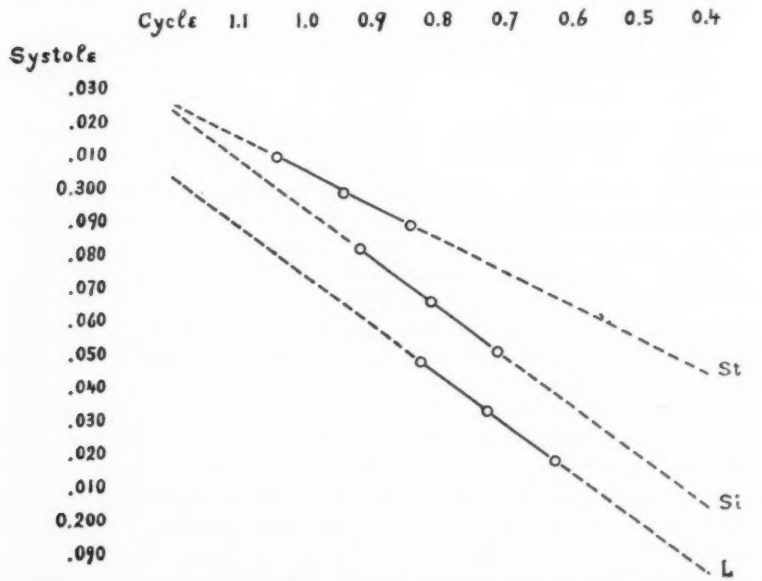

Fig. 8. Curves which show the effect of position on the average duration, and rate of change of length, of the systoles of normal men, for different cycle lengths, the duration of the systoles being the ordinates, and the cycle lengths the abscissae. *St.*, standing; *Si.*, sitting; *L.*, recumbent.

the subjects after they had run twice up and down a flight of stairs. The effect of exercise is being made a subject of special study, and we hope to report upon it later.

We determined the curves of the average length of the systoles of men (see fig. 8) and women when sitting and lying down, in the way we have just described for the curve of the systoles of men standing. The data used in plotting the curve of the systoles of men are given in table 1, and of women in table 2.

Using tables 1 and 2, we have constructed formulae which permit one to calculate the average length of the systoles of men and women, in the standing, sitting, and recumbent positions, for all ordinary cycle lengths. These formulae were constructed according to the general equation for a straight line, viz., $S = aC + b$, in which S = length of systole in seconds, C = length of cycle in seconds, a = the slope, and b = the height of the curve:

Men, standing at rest:.....$S = 0.150\ C + 0.1242$
Men, sitting.....$S = 0.147\ C + 0.1478$
Men, recumbent.....$S = 0.105\ C + 0.2010$

Women standing at rest.....$S = 0.157\ C + 0.1375$
Women, sitting.....$S = 0.135\ C + 0.1683$
Women, recumbent.....$S = 0.065\ C + 0.2478$

In this connection, mention should be made concerning the formulae which have been constructed by others to give the duration of the systole, at different pulse rates. Garrod (13) gave the following formula for systole length of a subject lying down: $xy = 20\sqrt{x}$, in which x = heart-rate, and y = the relation of systole to cycle. Waller (14) gives a formula $S = 0.343\sqrt{C}$, using the same nomenclature as we do. Fridericia (15) states: $S = 8.22\sqrt[3]{p}$, in which p denotes the electrical systole. Bazett (11), using the electrocardiogram, gives the following formula: $S = k\sqrt{C}$, in which k has a value of 0.37 for men, and 0.40 for women. We have found none of these formulae to be satisfactory, inasmuch as they all express the systole as being either a square root or cube root function of the cycle, while our data point to a straight line formula as expressing the relationship between the systoles and cycles for ordinary pulse rates of men and women at rest. We have only a very few cases of pulse rates higher than 120, but the systoles in these cases apparently are shorter than our formula would indicate, and the plot of figure 7 which we made, superposing the average duration of the systoles in groups of cycle lengths covering 50 cycles, suggests that the straight line fall of the curve may not hold for short cycles. The number of our observations at such quick pulse rates in the case of men standing at rest was too small to permit a definite conclusion to be drawn. The matter will be referred to again on page 287.

From straight line formulae for men, and the curves shown in figure 8, we have been able to construct table 3, which enables one to compare the average length of systoles of men in the three positions, for cycle lengths differing by 0.100 second, for cycles from 1.200 to 0.300 second (pulse rates 50 to 200). Of course we did not have enough actual experimental data to give reliable averages for all the cycle lengths tabulated. Those marked * have been calculated on the assumption that the straight line

fall of the curves of figure 8 would continue beyond the range of the systoles observed in considerable numbers of cases which is doubtful.

The curves shown in figure 8 enable us to observe the effect of cycle length on the duration of the systole for each of the positions in which the tests were made, and the relative duration of the systoles for like cycle lengths in different positions.

TABLE 3

Effect of position on the average length of the systole for different pulse rates in the case of men. The duration of the systole is stated in seconds

CYCLE LENGTH	PULSE RATE	STANDING	SITTING	RECUMBENT
1.200	50	*0.3042	0.3242	0.3270
1.100	54.5	0.2892	0.3095	0.3165
1.000	60	0.2742	0.2948	0.3060
0.900	66.6	0.2592	0.2801	0.2955
0.800	75	0.2442	0.2654	0.2850
0.700	85.7	0.2292	0.2507	0.2745
0.600	100	0.2142	0.2360	0.2640
0.500	120	0.1992	*0.2213	*0.2535
0.400	150	*0.1842	*0.2066	*0.2430
0.300	200	*0.1692	*0.1919	*0.2325

The values marked * were calculated on the assumption that the rate of change of length of systole would be maintained outside the limits of actual observation.

The following is a summary of the facts brought out by the curves: A. The relation of the average duration of the systoles to the duration of the corresponding cycles of individuals at rest may be expressed as a straight line, the pitch of which indicates the rate at which the systoles shorten with decreasing cycle lengths.

B. The relative height of the curves shows the effect of the position of the subject on the length of the systoles, the systoles being longer in the recumbent than in the sitting, and in the sitting than in the standing position.

C. The relative pitch of the curves indicates that with decreasing cycle lengths the systoles shorten a little less rapidly in the sitting than in the standing, and much less rapidly in the recumbent than in the sitting position.

We believe that this summary of the character, relative height and relative pitch of the curves is in general correct. One can offer as evidence of this the fact that, in spite of the differences found for the height and pitch of the curves of the systoles of men and women, the effect of position on the relative height and relative pitch is the same for both sexes. An explanation of the above facts is difficult, and is offered only as a discussion of the results of our observations. Before we give this explanation, we wish to draw attention to a few general considerations.

Some general considerations. Our experimental work dealt only with the duration of the systole of the left ventricle; but if more or less blood comes to the right ventricle, the left ventricle will have to handle a corresponding amount of blood in a very short time, and the average length of the systoles of the left ventricle will be influenced by the quantity of blood supplied to the right heart. It is true that the length of the systole is influenced by respiratory changes, and to a less extent, by vasomotor influences, but as this paper deals with the *average* duration of the systoles, we shall postpone reference to these transient influences until we can devote more space to them than would be possible in this paper.

When the pulse rate quickens, cycles shorten because of decrease both in systole and diastole length. The end of the diastole and the beginning of the next systole are determined by impulses from the sino-auricular node, but the termination of the systole and the beginning of the following diastole must be brought about by other influences.

The systole is a muscular contraction, and its duration must depend primarily on the internal contraction processes characteristic of ventricular muscle. It is known that these processes in the mammalian heart are modified by the temperature and the chemical constitution of the blood, and by the mechanical stretching of the muscle fibers by the venous blood during diastole, and to a certain extent, at least, by the action of nerves. It is unlikely that the differences which we observed in the duration of the systole were due to changes in thermal or chemical influences. From the work of others on animals, it would appear that the vagus nerves have little direct effect on ventricular muscle, although some believe that there is a tonus of the heart muscle which is influenced; the accelerator nerves, however, seem to play an important part. Wiggers and Katz (25) conclude from their experiments on dogs that, "the accelerator nerves have a specific effect on the ventricular musculature which operates to reduce the contraction period." Some possible confirmation of this conclusion is suggested by the results which we obtained from measuring the length of systoles of subjects with high pulse rates, especially of those standing immediately after exercise. So far as this study is concerned, we must be content merely to state that we recognize this possibility. Certainly most of the differences which we have observed on subjects at rest are best explained as due to the mechanical effect of the volume and pressure of the venous blood supplied to the heart.

There is much evidence that the duration of the systole is influenced by the quantity of blood collecting in the veno-auricular reservoir between beats and acting on the ventricular musculature at the instant it begins to contract.

a. It is true of the heart of the frog, as seen in laboratory experiments demonstrating the refractory period and the so-called "compensatory pause." If the artificial stimulus falls during early diastole and calls out

an extra contraction, the auricle beat occurring at this time finds the heart refractory, fails to excite a contraction, and is followed by a period during which the ventricle waits for the next auricular stimulus. The extra contraction is usually incomplete, and the next systole of the ventricle has to handle an unusually large amount of blood—that left over from the two preceding cycles—and is unusually large, and occupies a longer time than those which precede and follow it. Indeed, it is often so much prolonged that the next auricle beat may come before the relaxation of the ventricle is complete. If this happens, the ventricle contracts again before it has received its ordinary supply of blood, and the next systole is unusually short. It may happen that these phenomena repeat themselves for several beats, and a sort of "pulsus alternans" is observed, until the volume of the blood to be handled per beat has become adjusted, and the systoles get back to their ordinary length.

b. In a similar manner, if an extra incomplete beat is interpolated in the pulse curve of an otherwise regularly beating human heart, and is followed, as is often the case, by an unusually long diastole, the interpolated beat not having pumped much blood, the following systole is seen to be lengthened, because of the unusual amount of blood in the veno-auricular reservoir and ventricle.

c. A number of observers (12), (22), (26) have studied the effect of the volume of the venous return on the hearts of animals, by injecting saline solutions into the veins, and have seen the systoles to be lengthened. Among others, R. Tigerstedt (27) has called especial attention to the relation of the filling of the central veins to the circulation.

d. The effect of the accumulation of venous blood during slow heart rates shows itself when the heart is slowed by artificial stimulation of the vagus nerve. Some years ago, Lombard and Budgett (28) recorded the pulse beats of the carotid artery of a rabbit under chloretone, by a lever attached to the central stump of the artery, when it was tied off and divided. The lever was the lightest compatible with stiffness (a film of bamboo, with a delicate paper writing point), and gave very little throw. The records pictured the result of the longitudinal expansion and recoil of the artery, and gave the best sphygmograms that one can imagine obtained by purely mechanical means. The duration of the systole, as measured from the beginning of the primary upstroke to the bottom of the dicrotic notch, could be determined with great accuracy. In these experiments, stimulation of the vagus lengthened both systole and diastole. As the vagus, probably, has little effect on the action of the ventricle, the lengthening of the systole was due to the increased venous filling during the prolonged diastole, much blood having accumulated during the lengthened cycles. Incidentally, it may be mentioned that we found the following note, accompanying the record of October 3, 1903: "The after-effect of

the vagus is different for systole and diastole. Diastole recovers much sooner than systole." Following excitation of the vagus, diastole shortens as the rate quickens to the normal, faster than systole, which is still under the influence of the unusual amount of venous blood. This is of interest because it corroborates by experiments on a rabbit, what Wiggers (22), also Katz (12), have found in the case of a dog. v. Frey and Krehl (29) ascertained that the total positive pressure phase of the ventricle, though not changed very much (to 30 per cent), may be lengthened by various conditions, such as massage of the abdomen and slowing of the heart by vagus excitation, which increase the filling of the ventricle.

We shall now attempt to explain the character of the curves, and the relative height and pitch of the curves of figure 8.

A. At ordinary pulse rates, the relation of the average duration of the systoles of men at rest, to the duration of the corresponding cycles, can be expressed as a straight line, the pitch of which indicates the rate at which the systoles shorten with decreasing cycle lengths.

1. Relative duration of cycles, systoles and diastoles, of men standing. One might expect the systole to occupy a definite proportion of the cycle at all pulse rates. This is not the case, however, because the length of the cycle is determined more by the length of the diastole than of the systole, and these do not vary by like amounts. In the case of men standing at rest, for every decrease of cycle length of 0.100 second, *at ordinary pulse rates*, the systole shortens 0.015 second, and the diastole 0.085 second, i.e., both the cycles and the diastoles shorten faster than the systoles, and the systoles come to occupy an ever increasing proportion of the cycles. This relation is to be seen in table 4, which gives the percentages of the cycles occupied by the systoles and diastoles with each succeeding shortening of 0.100 second in cycle length, as estimated from carotid pulse curves.

The percentage change of the average systoles and diastoles with respect to the duration of the average cycles is the same, but the change takes place in opposite directions, the diastole occupying smaller, and the systoles larger proportions of the cycles, as the cycles shorten.

Eyster (17) studied men in the recumbent, and Brugsch and Blumenfeld (30) in the standing position. Their methods were different from ours, and they estimated the systoles to occupy larger proportions of the cycles.

As the systoles shorten less rapidly than the cycles, with increasing pulse rates, the systole-cycle ratio, $\frac{\text{systole}}{\text{cycle}}$, increases more and more rapidly as the cycles shorten. Table 5 gives the systole-cycle ratio for different cycle lengths and different positions, in the case of men, as measured on carotid sphygmograms.

If one compares the systole-cycle ratios for the different positions, it is found that the systole-cycle ratio is larger for all cycle lengths in the case

of sitting than standing, because the blood is returning to the heart more rapidly in the case of sitting than standing, and the systoles occupy a relatively larger proportion of the cycles. The same thing is true to a still greater degree in the case of the recumbent position, the systole-cycle ratio being larger in the case of lying than sitting, and still larger in lying down than standing, for all cycle lengths.

TABLE 4

Percentage of cycle occupied by systole and diastole, in men, standing at rest

CYCLE LENGTH	SYSTOLE	DIASTOLE	PULSE RATE
seconds	*per cent*	*per cent*	
1.200	25.35	74.65	50.0
1.100	26.29	73.71	54.5
1.000	27.42	72.58	60.0
0.900	28.80	71.20	66.6
0.800	30.53	69.47	75.0
0.700	32.74	67.26	85.7
0.600	35.70	64.30	100.0
0.500	39.84	60.16	120.0

TABLE 5

Systole-cycle ratios for different positions and cycle lengths

CYCLES	SYSTOLE-CYCLE RATIO		
	Standing	Sitting	Recumbent
seconds			
1.200	0.253	0.270	0.272
1.100	0.263	0.281	0.288
1.000	0.274	0.295	0.306
0.900	0.288	0.311	0.328
0.800	0.305	0.332	0.356
0.700	0.328	0.358	0.392
0.600	0.357	0.393	0.440
0.500	0.398	0.443	0.507

2. What governs the decrease in the duration of the systoles as cycles shorten with increasing pulse rates?

a. The quicker the heart rate, the more rapidly the blood is pumped out of the veno-auricular reservoir, and the less the amount of blood which is left from the preceding cycles.

b. As the heart rate increases, the total cycle, the period during which blood can collect on the venous side of the heart, shortens, and the amount and pressure of the blood in the veno-auricular reservoir decreases in spite of the fact that at quicker pulse rates the circulation of the blood is more

rapid. At ordinary pulse rates, it is the cycle length rather than the length of the diastole which influences the length of the systole.

The progressive effect of these combined factors acts, as the cycles shorten, to cause the duration of the systoles to lessen in a regular manner, so that the curve of the duration of the systoles plotted against cycle lengths is a straight line, for ordinary cycle lengths of men at rest. (For the effect of very rapid pulse rates, see 4, page 287.)

3. Diagram showing the relative duration of the phases of the heart cycle of man, standing, with increasing heart rates.

The relation of the length of the systoles, diastoles, and periods of diastasis, to each other, and to the cycle lengths, with increasing pulse rates, in the case of men standing at rest, can be pictured by a diagram such as is shown in figure 9. The following values were used in constructing the diagram: At pulse rate 60, the duration of the cycle is 1.000 second; of the systole, 0.270 second; and of the diastole, 0.730 second. At pulse rate 120, the duration of the cycle, systole, and diastole are respectively 0.500, 0.200 and 0.300 second (see table 3).

As has been emphasized, the carotid pulse does not give the true duration of systole, but it gives with considerable accuracy the interval between the rise of the primary wave and the rise of the dicrotic wave, the P–D time. The true duration of the systole may be calculated by subtracting the time required for the fall of the descending limb of the dicrotic notch from the isometric period, and adding the difference to the P–D time. Unfortunately this cannot be done for the standing position, because the isometric period is not known for this position. If the isometric period is on the average 0.076 second (Robinson and Draper, 8), in the recumbent, and 0.050 second (Wiggers and Clough, 16), in the sitting position, it is probably still less in the standing position. As the time required for the fall of the descending limb of the dicrotic notch averages 0.035 second, in the standing position, the difference between that and the isometric period would be so small that one can assume that the P–D time closely approximates the duration of the systole in the standing position. The period of relaxation of the ventricle preceding the opening of the auriculo-ventricular valves, the period of rapid filling, and the duration of the contraction of the auricle, were each assumed to occupy an average time of 0.100 second, there being no exact data for the length of these phases of the cycle, with changing cycle lengths, available in the case of man. Burstein (31) found wide variations in the periods of relaxation and of rapid inflow, with averages a trifle larger than the values we assume, but in the two sphygmograms which he shows in his figure 1, with cycle lengths of 0.960 and 0.950 second, the periods of relaxation and rapid inflow differ not more than 0.004 second, and for the most part are a little less than 0.100 second. For the purpose of our diagram, the figures which we give are sufficiently accurate.

The diagram will serve to illustrate the following points:

a. Cycles, systoles, and diastoles shorten at different rates.

b. Diastoles shorten much faster than systoles, as the cycles shorten.

c. The length of the long cycles, at least, is determined much more by the duration of the diastoles than the duration of the systoles.

d. As the cycles shorten, the systoles occupy an ever increasing proportion of the cycle.

e. The period of diastasis rapidly lessens as the cycles shorten, and consequently the period of rapid filling, and the filling caused by the contraction of the auricles becomes of greater and greater importance as the pulse becomes more rapid.

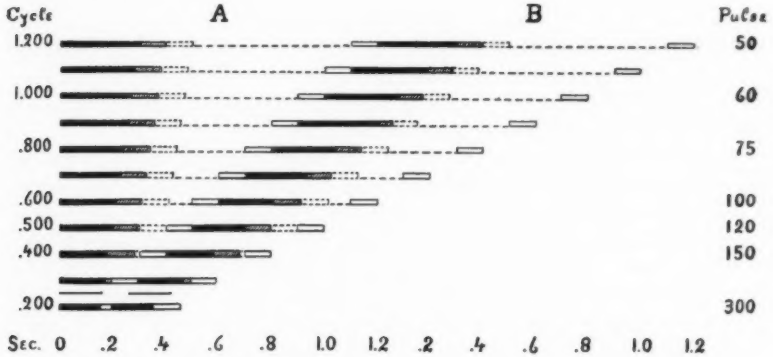

Fig. 9. Diagram showing the relative duration of cycles, systoles, and diastoles, of men standing, with increasing pulse rates. Two succeeding cycles are pictured. The cycle length in seconds, is placed to the left, and the pulse rate to the right. At the bottom, the time is given in seconds. The broad, heavily shaded portion indicates the duration of systole; the lightly shaded region, the period of relaxation preceding the opening of the auriculoventricular valves; the space enclosed by the two broken lines, the period of rapid filling of the ventricle; the interval covered by the single broken line, the period of diastasis; the space enclosed by the two unbroken lines, the systole of the auricle. The relative importance of the period of rapid filling, and of the contraction of the auricle, for the filling of the ventricle in rapid pulse rates is evident.

f. At cycle length about 0.500 second, pulse rate 120, there would be no period of diastasis, and the whole of the filling of the ventricle would occur during the phase of rapid filling and the contraction of the auricle.

g. At some shorter cycle length—0.382, pulse rate 157, according to our diagram—(although we are not so sure of the figures at cycle lengths shorter than 0.500 second, (see p. 278), there would be no phase of rapid filling of the ventricle, and the filling would be produced wholly by the contraction of the auricle.

h. At a still shorter cycle length—0.264 second (?), pulse rate 227 (?), the auricle would begin to contract at the instant the systole of the ventricle ceased, and later yet, the systole of the auricle would start before ventricular systole ceased.

In this connection attention should be called to the work of Gesell (32) on the heart of the dog, in which auricular systoles were made to occur in different parts of the ventricular cycle. He found not only that "Auricular contractions play an important rôle in determining the ventricular output," but "Auricular systoles partly stoppered by ventricular systoles have a positive effect on the filling of the ventricles."

It was not until the diagram of figure 9 had been worked out to show the effect of quickening of the heart rate on the filling of the ventricle of man, that we came upon Wiggers' (22) estimate of the effect of the brief diastoles of rapid heart rates on the method of filling of the ventricles of the dog. His figures are, of course, quite different from ours, as the time intervals of the different phases of the heart action of the dog are considerably shorter than those of the human heart.

Yandell Henderson and Barringer (33) have rendered valuable service by their analysis of the conditions which determine the filling of the ventricle of the animal heart, even though some of their conclusions have not been substantiated by the work of most of the later investigators in this field (25), (34). As a result of our study of the human heart, we have been led to believe that there is, as Henderson emphasized, a rapid rush of blood into the ventricle at the instant that the auriculo-ventricular valves open. At ordinary pulse rates this only partly fills the ventricle, however, and during the period of diastasis blood continues to accumulate in the ventricle to a greater extent than Henderson implies by the term "diastasis." Although the rate of beat determines the length of the cycle, and therefore the time during which blood can accumulate in the veno-auricular reservoir, the factors which determine the rate and volume of flow of venous blood toward the heart are of no less importance, and the output of the heart, as well as the length of the systole, is determined by no means wholly by the rate of heart beat. As Zuntz (34) decided, the heart is capable of varying the amplitude of its stroke enormously independently of the rate of beat. The effect of position of the subject, which we first pointed out in 1919, gives striking proof of the correctness of this view, and the same would be true of any condition which influences the return of venous blood to the heart. Thus our results are opposed to those of Henderson and Barringer (33), who conclude, "The systolic discharge under normal conditions (i.e., with an adequate venous pressure and supply) and at slow rates of beat is for the individual a practically unvarying volume," unless they refer to excessively slow rates of beat, when the heart would take in all the blood it could hold. Although Henderson's principle—

"Uniformity of behavior"—may be all right for the isolated heart under the conditions with which he worked, it does not appear to apply to the human heart working as it does under the varying conditions of venous flow, volume and pressure.

4. When cycle lengths shorten to give pulse rates higher than those ordinarily occurring during rest, it is possible for new conditions to arise, which would cause the straight line fall of the curve to change to a more and more rapidly descending curve.

a. One of these conditions would be the *shortening of the period of rapid filling of the ventricle* by the encroachment of the beginning of the contraction of the auricle. The blood which has been accumulating in the auricle during the contraction of the ventricle, rushes into the ventricle the instant the auriculo-ventricular valves open. During the diastasis immediately following, blood continues to accumulate in the veno-auricular reservoir, and to flow into the ventricle, especially during long cycles. But as the heart rate increases beyond the ordinary resting values, the cycles shorten until the phase of diastasis disappears, and the phase of rapid filling is encroached upon by the beginning of the contraction of the auricle. (See diagram, fig. 9.) The shortening of the period of rapid filling would apparently begin to be felt in the case of man in the standing position at a cycle length of about 0.500 second—pulse rate 120.

It is interesting to recall that Meek (26) studied the output of the heart of the dog under ether, by observing the diastolic size of the heart as shown by the x-ray. He found a slow decrease of the output as the rate changed from 50 to 110 per minute, but above 110 a rapid decrease. He explained this on the basis that the phase of rapid filling of the ventricle is encroached upon.

b. Wiggers and Katz (26) have found that *the accelerator nerve of the dog has a direct effect to lessen the contraction period.* The same may be true of the human heart, when the pulse rate is driven up by the action of that nerve. Such an effect should be of value because it would slightly lengthen the time during which blood could enter the ventricle when the heart rate is very rapid. Their measurements, as well as those of Wiggers and his associates (9), of the ejection phases of the human heart, correspond with Meek's observation above referred to, in making it probable that at pulse rates higher than those ordinarily observed in the resting man, the systoles would be shorter than a straight line fall of the curve of systoles plotted against cycle lengths would indicate.

These views are borne out by the length of the systoles which we have determined from the records of men with high pulse rates standing just following exercise. Although one of us expects to report later on the effect of exercise on the length of the systole, it may not be out of place to state here that we plotted the curves of the duration of the systoles (cycles being

the abscissae), of men standing after resting and after exercise, and found that the curves crossed, at cycle length about 0.500 second, the exercise curve being higher at longer, and lower at shorter cycle lengths, than the straight line curve of systoles of men resting. One would expect that the larger volume of returning venous blood just following exercise (Hooker and Schneider (35) found the venous pressure to be increased just after exercise), would tend to make the systoles longer than they would be in the same cycle lengths just after resting. There seems to be no reason, however, why the systoles should be shorter after exercise than after resting in like cycle lengths, as suggested by the apparent crossing of the curves at cycle length 0.500 second. It is at about this part of the curve that our knowledge of the duration of the systoles of men standing after resting is lacking, and it is not unlikely that at about this point the straight line fall of the curve of the duration of the systoles fails, and that the true curve tends to fall more and more rapidly. As has been said it is at pulse rates above 120 that the period of rapid filling begins to be encroached upon by the beginning of the auricular systoles, and moreover, the accelerator may shorten the systoles at the same time that it quickens the pulse rate. It is probable, therefore, that in cycle lengths shorter than 0.500 second the curve of the duration of the systoles in the standing position, plotted against cycle lengths, may change its course from a straight line fall, and approach a $\sqrt{}$ or $\sqrt[3]{}$ curve. Several investigators have offered such curves for the whole course of the shortening of the systoles under the influence of quickening pulse rates. (See page 278.)

B. The relative height of the curves of figure 8 shows the effect of position of the subject on the length of the systoles, they being longer in the recumbent than in the sitting, and in the sitting than in the standing posture.

The duration of the systole is influenced not only by the pulse rate whatever the position of the body, but by the position of the body whatever the pulse rate. The fact that the length of the systole is influenced by the position of the body has failed to attract the attention of most observers. Indeed, most of those who have written concerning the duration of the systole, although recognizing the importance of pulse rate, failed even to state the position of the subjects tested in their experiments. Donders (36) made some observations on one man that suggested that the systole length is different for sitting as compared with standing. Garrod (13) also noticed in work on himself, that it was different for standing, sitting, and lying down. Our results confirm these observations.

The fact that the curves taken in different positions are at different levels, is undoubtedly due to the effect of gravity, which would oppose the return of venous blood from the abdomen, legs and arms in the standing position, and from the abdomen, lower legs, and upper arms, in the sitting posture.

To test the effect of gravity, we made a few experiments on men lying

on a tilting board, thus causing the large veins at the heart to be higher or lower than those of the abdomen and the lower limbs. The amount of tilting produced was not sufficient to cause the subject to make muscular efforts to retain his position. In one of these experiments we obtained the following results:

No. 126a. Head up 40°, the systole was 0.049 second shorter than the average, at the same pulse rate, in the horizontal position.

Head down 15°, the systole was 0.022 second longer than the average, at the same pulse rate, in the horizontal position.

The clinical effects of gravity have received considerable attention as will be seen by a study of the work of such men as v. Frey (38), Hill (39), Sewall (40), Crampton (37) and Hooker (35), but as stated above, none of them have called special attention to the effect of gravity on the length of the systole of men.

C. The relative pitch of the curves indicates that as cycle lengths decrease the systoles shorten less rapdily in the sitting than in the standing, and still less rapidly in the recumbent posture.

The difference in the pitch of the curves in the three positions brought out in figure 8 and the formulae, p. 278, is, as has been said, due to the effect of gravity on the return of the venous blood to the heart. In long cycles (slow rates), there is time for the blood to get back to the heart in spite of the retarding effect of gravity in the standing and sitting positions; but as cycles shorten, gravity has a progressively greater influence, and although the effect of short cycles on the accumulation of venous blood is to be seen in all positions, it is more marked in the sitting, and still more in the standing curve.

If the curves of figure 8 were prolonged, those of the standing and sitting position would cross those of the recumbent posture. This crossing of the curves is an apparent absurdity. We have no experimental data with regard to the length of the systoles of abnormally long cycles. Probably, in such cases, the blood would tend to collect in the large veins near the heart, and it would receive large amounts of blood in all positions, the effects of gravity being largely compensated for by the long cycles. In other words, it is not unlikely that in abnormally long cycles the heart would receive the maximal amount of blood it could handle, in each of the positions, and the systoles would be about the same length in all positions and for all individuals.

Effect of arterial pressure on the length of the systole. It might be thought that the length of the systole would be influenced by the resistance encountered in driving the blood into the arteries. To test this factor, we measured the blood pressures of 86 men while sitting after resting, by the Korotkow method, just before the carotid pulse was recorded. While the blood pressures and the duration of the systoles differed considerably

in different men, we could find no correspondence between the systolic, diastolic, and pulse pressures and the length of the systoles, when these were plotted as ordinates, with corresponding cycle lengths as abscissae. A tabulation of these factors in the case of 36 men who had systolic pressures above 126 mm., showed that the systoles were in some cases longer and in others shorter than the standard average systole lengths of the cycle in question, regardless of the blood pressures of the men tested. Although we had expected to find a relation between the duration of the systole and the blood pressure, we were forced to conclude that in the case of normal men with ordinary ranges of blood pressure, no constant relation exists. Our results are in harmony, therefore, with those of von Frey and Krehl (29) and Hürthle (41). They apparently differ from those of Straub (34), who concluded that "Die Dauer der Austreibungszeit wächst mit zunahme der Ueberlastung und mit zunahme des Schlagvolums;" also Patterson, Piper and Starling (34), "Increase in arterial resistance and in venous return increase the duration of the systole." Wiggers reports that increase in arterial pressure acts to abbreviate systole independently of changes in diastole, but that this tendency may be overcome by increased initial tension. We did not observe the effects of very high arterial pressures, but rather the factors which the heart of a normal man encounters when acting under the stable condition of the circulation of a man at rest. Our results are probably explained by the "Law of the heart" enunciated by Patterson, Piper and Starling (34), the effect of arterial resistance being more or less completely compensated for by the resulting initial tension, in Wiggers' sense, and the compensatory stretching of the heart fibers.

If one considers the height of the arterial blood pressure, and the duration of the systoles, in different positions, he recognizes that in the recumbent posture the arterial pressure is relatively low and the systoles long, while in the standing position, the blood pressure is relatively high, and the systoles short. The difference in the pressure is ordinarily a few millimeters, however, and cannot account for the differences in the length of the systoles, which is due, as has been explained, to the effect of gravity on the return of the venous blood to the heart. There is little doubt that the long systoles imply that the volume output per beat is large, which would suggest that the blood pressure should be high in the recumbent position, but the pulse rate is slow, which causes the blood pressure to be low. In a similar manner, the relatively high pressure in the standing position, when the systoles are short, and stroke-volume small, is to be explained, other factors being the same, by the higher pulse rate.

Effect of other influences on the duration of systole. One might expect to find that the duration of the systole would be influenced by other conditions beside heart rate and the position of the subject. We can only say that

we failed to find any correlation between the length of the systole, and age (17–66 years), height, weight, or physical training; also no differences in the duration of the systoles of smokers and non-smokers.

Normal deviations from the standards established by this research. We have attempted to determine the average duration of the systole of men in the standing, sitting, and recumbent positions for all ordinary cycle lengths, and to establish formulae which can be used as standards with which the systoles of individuals may be compared. We have stated that the duration of the systoles of the men we have studied, differed in some cases as much as 0.025 second, plus or minus, from the standards. This maximum deviation is very large, and it is desirable to know what is the ordinary extent of the deviation to be expected. This is stated in the following table in percentages of the men tested.

DEVIATION FROM STANDARD	STANDING	SITTING	RECUMBENT
Not more than 0.005 second	35.5	35.8	40.0
Not more than 0.010 second	66.9	67.0	70.8
Not more than 0.015 second	84.9	86.1	84.6
Not more than 0.020 second	94.8	92.5	98.5

A difference of 0.025 second from the standard values would be a larger proportional difference in the case of higher than lower pulse rates, also in the case of the shorter systoles peculiar to the standing position than the long systoles of the recumbent posture. Nevertheless similar differences are observed for all pulse rates and in all positions.

It is also of interest to know what deviations from the standard may occur if the length of the systoles of the same man are tested at different times. Sixty-three men were examined in the standing position on two days, and the deviation of the recorded systoles from the standard were about the same as those observed on different men.

We cannot decide how far the deviations from the standards which we have established for the average duration of the systoles of normal men are merely due to errors, or are physiological differences. Only a small part can be attributed to errors in the reading of the curves, because very frequently curves were read by both of the writers and it was rare for us not to agree within 0.002 second. On the other hand, the decision of the exact point where the pulse curve begins to rise, and what shall be regarded as the bottom of the notch, is a matter of judgment, and may be a source of error. Moreover, the variations in the time required for the fall of the descending limb of the dicrotic notch as compared with the isometric period, may be responsible for some of the variations. (See p. 266.)

On the whole, we are not so much impressed by the deviations from the standards, as the fact that the variations are not greater. They must

cover not only the errors of the method, but differences due to the rate at which the venous blood returns to the heart under the varying conditions of the peripheral, and especially of the abdominal vessels, and the size of the heart with respect to the quantity of blood to be pumped. It is possible, although doubtful, that the tonus of the heart may alter the amount of blood the ventricle can accommodate, or that the nerves may in some way affect the rate and completeness of the ventricular contraction. Finally it is possible that other as yet unknown influences may act to change the efficiency of the heart.

Conditions influencing the minute-output and stroke-volume of the heart. The relative importance of the heart rate and the amplitude of the stroke for the output per minute has been the subject of much study. For the literature one may refer to papers by R. Tigerstedt (42), Henderson (33) and Wiggers (43). Most observers have recognized that the arterial blood stream is subject to great fluctuations, that the amplitude of the beat plays as important a rôle as the heart rate, and that these may vary independently. Henderson, however, differs, concluding that when the venous supply to the right heart is ample, the rate is the fundamental variable, the amplitude being dependent on the rate. An admirable discussion of the whole question is given by Wiggers. Most of the work was done on the hearts of animals, and any indication concerning the behavior of the human heart would be of value.

The length of the systole is so definitely influenced by the quantity of the venous blood supplied to the heart of man, that the duration of the systole can be considered as giving a valuable indication of the amount of venous blood supplied to the heart, and the amount the heart pumps per beat. When the rate of beat is increased, the amount of blood pumped by the animal heart per beat is less, and we find the systoles of men to be shorter; when the pulse is slowed, the output of the animal heart per beat is more, and we find the systoles of men to be longer. When the return of the venous blood is greater, the heart of the animal adjusts itself to pump more blood per beat, and we find the systoles of men are longer, the reverse being true when the venous return is lessened. If this view is correct, the results recorded in this paper give evidence that in the case of the human heart the conditions which control the return of the venous blood are no less important than the heart rate in determining the output of the heart per minute.

Apparently the duration of the systole—the P–D time—less the time required for the fall of the descending limb of the dicrotic notch as measured on the carotid curve, would give a figure for the ejection period, and this figure, multiplied by the pulse rate, would give, not a measure, but an indication of the volume output of the heart per minute. It is doubtful, however, whether these figures could be used as a standard of comparison

for the hearts of all men, and still more for the hearts of both men and women, because the size of the heart in relation to the amount of blood it is called upon to care for would probably play a rôle. At least, it would be interesting to compare the length of the systole, or better the ejection period, of the left ventricle of man in different positions, as determined by the carotid pulse curve, with the "stroke index" as determined by the ethyl iodide method recently described by Yandell Henderson and H. W. Haggard (44). They report that the stroke index, or the volume in cubic millimeters of blood per kilo of body weight discharged by the heart per beat, for normal persons during sitting rest, ranges from 1.3 to 1.8, and in some individuals is much larger in the recumbent position—up to 2.0 or more; and much smaller in the standing position—down to 1.0 or less. They say that these observations confirm those of Bock and Field. During vigorous exercise the stroke index may rise to about 3.5.

SUMMARY

1. In this paper the word systole is used for the time between the beginning of the rise of the primary wave of the carotid sphygmogram and the bottom of the dicrotic notch, an interval which may be called the P–D time. The difference between the duration of the isometric period and the time required for the fall of the descending limb of the dicrotic notch, would have to be added to the P–D time, which we measured, to obtain the duration of the true systole. This difference is unknown, but probably is very slight, in the standing position, and somewhat greater in the sitting and the recumbent postures.

2. The length of successive systoles is so variable, that at least fifteen cycles must be measured to obtain a reliable average.

3. The length of the average systole has a definite relation to the length of the average cycle, in the case of a subject at rest, and this relation can be expressed by a straight line formula, but such a formula probably does not hold good for cycle lengths shorter than 0.500 second (pulse rates above 120), in the standing position, and probably for somewhat larger cycle lengths in the sitting and the recumbent postures. At these shorter cycle lengths, new factors influencing the length of systoles probably develop, causing them to lessen more and more rapidly as the cycles shorten. A diagram (see fig. 9), has been constructed to illustrate the relative importance of the periods of rapid filling, diastasis and auricular contractions in different cycle lengths.

4. The duration of the average systole, for like cycle lengths, is longer in the recumbent position than in the sitting, and in the sitting than in the standing posture, because gravity delays the return of the venous blood to the heart in the sitting, and still more in the standing position; therefore different formulae (p. 278) are necessary to express the relation of systole length to cycle length in different positions.

5. Because of the effect of gravity, the systoles become shorter, as the cycles shorten, more rapidly in the standing and sitting than in the recumbent posture. Slow pulse rates compensate for the effect of gravity, and the duration of the systoles tends to become about the same in very long cycles, in all positions. (See fig. 8.)

6. Henderson's "Law of uniform behavior" does not appear to apply to the human heart.

7. The length of the systoles of women is greater than of men for like cycle lengths in all positions; consequently different formulae have to be employed.

8. The length of the systole of the resting adult normal man or woman does not differ from the value which may be calculated from the formulae, more than 0.025 second, plus or minus. This is a large percentage difference, and it is to be explained to a considerable extent by variations in the circulatory conditions and the consequent rate and volume of the return of the venous blood to the heart.

9. The length of the systole could not be found to be influenced by the systolic, diastolic, or pulse pressure.

10. No relation was found to exist between the length of the systole and the time of day, the time of year, age, height, weight, or the smoking of tobacco.

11. The duration of the period of ejection of blood from the left ventricle can be measured with considerable accuracy on many carotid sphygmograms of men at rest. It can also be calculated for different cycle lengths and positions by subtracting from the P–D time, the systole as measured by us, the time required for the fall of the descending limb of the dicrotic notch.

In closing, we wish to express our indebtedness to Dr. A. L. Jacoby for the use of some of his curves; to Dr. R. Q. Brigham for his study on the effect of weight and height of the subjects; to Prof. W. P. Bowen for enabling us to secure many of the sphygmograms from women; and finally to a number of our friends who aided in our study of the formulae.

BIBLIOGRAPHY

(1) Sewall: Amer. Journ. Med. Sci., 1911, clviii, 786.
(2) Storey: This Journal, 1903, viii, 355.
(3) Lombard and Cope: Proc. Amer. Physiol. Soc., 1917,—This Journal, 1918, xlv, 564; Proc. Soc. Exper. Biol. and Med., 1919, xvi, 97; Proc. Amer. Physiol. Soc., 1918,—This Journal, 1919, xlix, 139, 140. Proc. Amer. Physiol. Soc., 1919,—This Journal, 1920, li, 174; Proc. Amer. Physiol. Soc., 1924,—This Journal, 1925, lxxii, 208.
(4) Wiggers and Dean: This Journal, 1917, xlii, 476; Amer. Jour. Med. Sci., 1917, cliii, 666.
(5) Bowen: This Journal, 1904, xi, 59.
(6) Marey: La Circulation du sang. Paris, 1881, 266.

(7) HÜRTHLE: Pflüger's Arch., 1891, xlix, 66.
(8) ROBINSON AND DRAPER: Arch. Int. Med., 1910, v, 206.
(9) WIGGERS: Circulation in health and disease. 2nd. ed., 1923, pp. 102, 206.
(10) TIGERSTEDT, R.: Physiol. des Kreislaufes. Berlin, 1921, 2nd. ed., i, 31.
(11) BAZETT: Heart, 1920, vii, 353.
(12) KATZ: Journ. Lab. Clin. Med., 1921, vi, 291.
(13) GARROD: Journ. Anat. and Physiol., 1870, v, 17.
THURSTON: Ibid., 1871, x, 494.
(14) WALLER: Introduction to human physiology, 1891, p. 53.
(15) FRIDERICIA: Acta med. Skand., 1920, liv, 17.
(16) WIGGERS AND CLOUGH: Journ. Lab. Clin. Med., 1919, iv, 624.
(17) EYSTER: Journ. Exper. Med., 1911, xiv, 603.
(18) LOMBARD AND PILLSBURY: This Journal, 1899, iii, 201.
(19) MACKENZIE: Study of the pulse. Young J. Pentland, 1902, p. 61.
(20) KEYT: Sphygmography and cardiography, physiological and clinical. 1887, 73.
(21) FRANK: Zeitschr. f. Biol., 1905, xlvi, 478.
(22) WIGGERS: This Journal, 1921, lvi, 415.
(23) GESELL: This Journal, 1911, xxix, 32 (19 authors cited).
(24) WIGGERS: This Journal, 1916, xl, 218.
(25) WIGGERS AND KATZ: This Journal, 1920, liii, 49.
(26) WIGGERS AND KATZ: This Journal, 1922, lviii, 450.
WIGGERS: Ibid., 1921, lvi, 424.
MEEK: Ibid., 1924, lxx, 385.
(27) TIGERSTEDT, R.: Finska Läkaresällskapets. 1918, p. 60.
(28) LOMBARD AND BUDGETT: Arch. internat. d. Physiol., 1904, 121.
(29) VON FREY UND KREHL: Arch. f. Anat. u. Physiol., Physiol. Abt., 1890, 51.
(30) BRUGSCH UND BLUMENFELDT: Berlin Klin. Wochenschr., 1919, lvi, 937.
(31) BURSTEIN: This Journal, 1923, lxv, 158.
(32) GESELL: This Journal, 1911, xxix, 62–63.
(33) HENDERSON: This Journal, 1906, xvi, 325; Ibid., 1909, xxiii, 345; Physiol. Reviews, 1923, iii, 195.
HENDERSON AND BARRINGER: This Journal, 1913, xxxi, 288, 352, 399.
(34) KNOWLTON AND STARLING: Journ. Physiol., 1912, xliv, 206.
KROGH: Skand. Arch. f. Physiol., 1912, xxvii, 126.
MEEK AND EYSTER: This Journal, 1922, li, 180.
PATTERSON, PIPER AND STARLING: This Journal, 1914, xlviii, 465.
PATTERSON AND STARLING: Ibid., 1914, xlviii, 357.
PLECH: Centralbl. f. Physiol., 1912, xxvi, 89.
STRAUB: Deutsch. Arch. f. klin. Med., 1914, cxv, 591; Ibid., 1914, cxvi, 409.
WIGGERS: This Journal, 1921, lvi, 439.
ZUNTZ: Zeitschr. f. klin. Med., 1912, 74.
(35) HOOKER: This Journal, 1911, xxviii, 235.
SCHNEIDER: Ibid., 1916, xl, 380.
(36) DONDERS: Neederl. Arch. voor Genees en Natur., 1865, ii, 139.
(37) CRAMPTON: Med. News, 1905, lxxxvii, 529; N. Y. Med. Journ., 1913, xcviii, 916; Amer. Journ. Med. Sci., 1920, clx, 721.
(38) VON FREY: Die Untersuching des Pulses, 1893, 214.
(39) HILL, L.: Journ. physiol., 1895, xviii, 15.
(40) SEWALL: Amer. Journ. Med. Sci., 1916, cli, 491.
(41) HÜRTHLE: Pflüger's Arch., 1891, xlvii, 17.
(42) TIGERSTEDT, R.: Ergebn. d. Physiol., 1905, iv, 481; 1906, vi, 265.
(43) WIGGERS: Arch. Int. Med., 1921, xxvii, 475.
(44) HENDERSON AND HAGGARD: This Journal, 1925, lxxiii, 193.

STUDIES ON THE PATHOGENESIS OF TETANY

V. The Prevention and Control of Parathyroid Tetany by Calcium Lactate

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In 1923, Luckhardt and Goldberg reported that they had kept dogs alive and in good condition following a complete thyro-parathyroidectomy by the oral administration of calcium lactate. They found it necessary in most cases to give very large doses approximating 1.5 grams of calcium lactate per kilogram of body weight per day to prevent the appearance of tetany or to relieve it. Subsequently Compere and Luckhardt (1924) reported that calcium carbonate, calcium nitrate and calcium acetate were as effective in relieving or preventing parathyroid tetany when given orally as was calcium lactate. Soluble monobasic calcium phosphate however seemed more liable to induce tetany than to relieve it.

It is the purpose of this communication to report data in some detail which confirm and extend the findings of Luckhardt and Goldberg (1923).

Preservation of life of adult thyro-parathyroidectomized dogs by the oral administration of calcium lactate. A complete thyro-parathyroidectomy was done in five adult female dogs. These were fed white bread, corn meal mush and water during the experiment. Calcium lactate dissolved in water or incorporated in corn meal mush was given by means of a large syringe and stomach tube.

Experimental results. Dog 70. Adult female. Weight, 6.4 kgm.

On April 24, 1924 a complete thyro-parathyroidectomy was done. From this date until May 6, 1924 animal remained free from tetany on 20 grams of calcium lactate given daily by stomach tube. May 6, tremors and spasticity appeared which were relieved by 20 grams of calcium lactate. From May 7 to 11 inclusive animal seemed normal. On May 12 tremors and spasticity again set in and occurred daily up to May 17 in spite of calcium lactate medication of 20 grams. From May 17 until October 31, 1924, the animal remained free from tetany symptoms. At this time pronounced bilateral cataracts were noted. From November 1 to July 17, 1925, this dog remained free from tetany without further treatment.

Dog 71. Adult female. Weight, 9 kgm.

On April 24, 1924, a complete thyro-parathyroidectomy was done. Twenty grams of calcium lactate were given daily by stomach tube throughout the experi-

ment. On April 26 severe tetany was noted from which the animal recovered after the daily dose of calcium lactate, but fatal tetany set in the following day in spite of calcium medication. No feces passed from this animal during the course of the experiment. At autopsy no accessary parathyroid tissue was found.

Dog 73. Adult female. Weight, 10 kgm.

On April 28, 1924, a complete thyro-parathyroidectomy was done. Twenty grams of calcium lactate were given daily, but in spite of this severe tetany developed on May 1, which required 50 grams of calcium lactate for relief. On May 2, at 8 a.m., moderate tetany developed and 50 grams of calcium lactate were given during the day is divided doses. From May 3 to 7 the animal remained free from tetany on 30 grams of calcium lactate. May 8 the daily dose of calcium lactate was reduced to 20 grams and the animal remained free from tetany and in good condition until 8 a.m. May 21, when a very severe attack of tetany suddenly occurred. During the attack, while attempting to pass the stomach tube, the dog collapsed and respiration ceased. Artificial respiration revived the animal and 50 grams of calcium lactate in divided doses were necessary to control the tetany. May 22, tetany again developed and 40 grams of calcium lactate in divided doses of 20 grams each were given. May 24, severe tetany was controlled by 40 grams calcium lactate. May 25, the animal died in tetany before treatment could be given. At autopsy no accessary parathyroid tissue was found.

Dog 74. Adult female. Weight, 4.5 kgm.

On May 5, 1924, a complete thyro-parathyroidectomy was done. On May 6 hyperpnea, tremors and spasticity appeared which were completely relieved by 20 grams of calcium lactate given by stomach tube. From May 7 to June 25, 20 grams of calcium lactate were given daily and animal remained free from tetany. From June 25 to 29 no calcium lactate was given and severe tetany occurred on June 30, which was again relieved by 20 grams of calcium lactate. This amount of calcium lactate given daily kept the animal free from tetany during July and on August 2 calcium medication was stopped and no further tetany occurred to date, July 17, 1925.

Dog 76. Adult female. Weight, 11 kgm.

On May 17, 1924, a complete thyro-parathyroidectomy was done. From May 18 to 21 inclusive tetany occurred daily in spite of the fact that 20 grams of calcium lactate were given daily. These attacks of tetany moderated to tremors and slight spasticity from May 22 to 24 inclusive under the same treatment. From May 25 to June 25 the animal remained free from tetany on 20 grams of calcium lactate. Calcium medication was stopped on May 26 and animal remained free from tetany and in good condition until July 28, 1925, when animal was used for other experiments.

It may be noted that of these five dogs, one died in tetany on the third day although 2.2 grams of calcium lactate per kilo of body weight were given daily. It is to be noted, however, that this dog passed no feces since the day of operation. Dragstedt, Phillips and Sudan (1923) pointed the importance of constipation as an exciting factor in parathyroid tetany and this finding is in harmony with those former observations. One dog died of tetany on the 28th day, although given 2 to 5 grams of calcium lactate per kilo daily. In the remaining three dogs, tetany was controlled and they survived in good condition on the following doses of calcium lactate respectively: 3.0 grams per kilo daily for 8 weeks, 4.4 grams per kilo for

11 weeks, and 1.8 grams per kilo daily for 5 weeks. In dog 74 tetany was controlled for 7 weeks by 4.4 grams of calcium lactate per kilo daily. No calcium was then given for 5 days when severe tetany appeared. Treatment was then constituted for 4 weeks longer before the animal could remain free from tetany without calcium medication. It should be noted that in all of these experiments the amounts of calcium lactate administered brought about the same change in the character of the feces that Dragstedt and Peacock (1923) found was produced by the oral administration of lactose. The feces became semi-fluid in consistency, light brown in color, relatively odorless, and acid to litmus.

Control of parathyroid tetany in pregnant dogs by the oral administration of calcium lactate. Dragstedt, Sudan and Phillips (1924) reported that pregnancy would induce severe and often fatal tetany in dogs that had recovered from a complete thyro-parathyroidectomy for long periods. This tetany of pregnancy could be relieved by the intravenous injection of large amounts of Ringer's solution or the oral administration of large doses of calcium lactate and lactose. In the following two experiments an attempt was made to conserve the life of pregnant dogs after a complete thyro-parathyroidectomy, by the oral administration of calcium lactate.

Experimental results. Dog 69. Adult female. Weight, 11 kgm. Pregnant.

Diet: corn meal mush, bread and water. On April 12, 1924, a complete thyro-parathyroidectomy was done. Twenty grams of calcium lactate in solution or incorporated in corn meal mush were given by stomach tube daily throughout the experiment. The animal remained in good condition and free from tetany from the date of operation until May 10, except on April 20 and 28 respectively, when tremors and spasticity were noted. These symptoms subsided after the daily calcium medication. On May 10 severe tetany (tremors, tonic and clonic convulsions, spasticity, hyperpnea and salivation) set in and proved fatal. At autopsy 8 fetuses near term were found in utero. No accessary parathyroid tissue was found.

Dog 72. Female adult. Weight, 10 kgm. Pregnant.

Diet: corn meal mush, bread and water. On April 28, 1924, a complete thyro-parathyroidectomy was done. From the date of operation until May 31 20 grams of calcium lactate were given daily by stomach tube and animal remained free from tetany. On June 1 the daily dose of calcium lactate was increased to 30 grams. On June 2 at 8:30 a.m. a slight uneasiness was noted which become progressively more marked until 9:00 a.m. when the animal developed severe tetany. The attack came on suddenly and was very severe, the convulsions being tonic from the first. These grew worse and respiration stopped at 9:15 a.m. Artificial respiration was administered and 10 cc. of a 10 per cent solution of calcium lactate were given intravenously but with no effect and the animal died in tetany at 9:20 a.m. A post-mortem examination done immediately disclosed 8 dead fetuses in utero. No accessary parathyroid tissue was found.

It is significant that 20 grams of calcium lactate daily sufficed to prevent tetany in both of these dogs for 29 and 35 days respectively, or until they were about to deliver, when a severe and fatal tetany appeared. Apparently the metabolic changes accompanying the latter part of pregnancy

and in particular the period immediately preceding delivery are more apt to induce tetany than early pregnancy. The convulsions observed in human eclampsia occur also more commonly in the latter part of pregnancy and immediately preceding and following delivery.

Control of parathyroid tetany in a young dog by the oral administration of calcium lactate. In harmony with many others, we have repeatedly observed that age is a very important factor in determining the severity of the symptom complex following complete thyro-parathyroidectomy in dogs. In young animals the onset of tetany or depression occurs much sooner, the symptoms are more severe, and death takes place more promptly than in adults. In aged animals the effect of thyro-parathyroidectomy is as a rule milder than in young adults.

In the following experiment the comparative difficulty of controlling tetany and conserving the life of a young male dog after complete thyro-parathyroidectomy is very evident. It is interesting to note that this animal was given white bread and milk ad libitum during the experiment and that in addition from 400 cc. to 600 cc. of milk were given daily by stomach tube. That milk alone cannot prevent tetany is evidenced by the fact that it required from 10 to 20 grams of calcium lactate or from 6 to 12 grams per kilo of body weight per day to control tetany in this animal, although given all the milk it could be forced to take.

Protocol. Dog 92. Male. Age 6 weeks. Weight, 1.7 kgm. Diet: Bread and milk ad libitum.

DATE 1925	TIME	REMARKS	AMOUNT OF CALCIUM LACTATE GIVEN ORALLY BY STOMACH TUBE
March 24	3:30 p.m.	Complete thyroparathyroidectomy	
25	1:00 p.m.	Spasm of orbicularis oculi	2.0 grams in 100 cc. milk
	4:30 p.m.	Tremors. Tetany expression	2.0 grams in 200 cc. milk
26	9:10 a.m.	Severe tetany. Respiration stopped in tonic convulsion when tube impinged on larynx	2.0 grams in 200 cc. milk
	1:30 p.m.	Condition good. No tetany	5.0 grams in 200 cc. milk
	4:30 p.m.	Condition good. No tetany	5.0 grams in 200 cc. milk
27	9:30 a.m.	Moderate tetany	5.0 grams in 200 cc. milk
	1:30 p.m.	Condition good. No tetany	5.0 grams in 200 cc. milk
	4:30 p.m.	Condition good. No tetany	5.0 grams in 200 cc. milk

DATE 1925	TIME	REMARKS	AMOUNT OF CALCIUM LACTATE GIVEN ORALLY BY STOMACH TUBE
March			
28	10:15 a.m.	Tremors. Tetany expression	5.0 grams in 200 cc. milk
	12:30 p.m.	Condition good. No tetany	5.0 grams in 200 cc. milk
	3:30 p.m.	Condition good. No tetany	5.0 grams in 200 cc. milk
29	9:30 a.m.	Slight tetany	5.0 grams in 200 cc. milk
	5:00 p.m.	Condition good. No tetany	5.0 grams in 200 cc. milk
30	9:30 a.m.	Spasticity	5.0 grams in 200 cc. milk
	3:00 p.m.	Condition good. No tetany	5.0 grams in 200 cc. milk
31	10:00 a.m.	Condition good. No tetany	5.0 grams in 200 cc. milk
	4:30 p.m.	Condition good. No tetany	5.0 grams in 200 cc. milk
April			
1	9:00 a.m.	Condition good. No tetany	5.0 grams in 200 cc. milk
2	9:00 a.m.	Tremors. Spasticity	5.0 grams in 200 cc. milk
	4:30 p.m.	Condition good. No tetany	5.0 grams in 200 cc. milk
3	9:00 a.m.	Condition good. No tetany	5.0 grams in 200 cc. milk
	4:00 p.m.	Condition good. No tetany	5.0 grams in 200 cc. milk
4	9:00 a.m.	Tremors. Spasticity	5.0 grams in 200 cc. milk
	1:00 p.m.	Condition good. No tetany	5.0 grams in 200 cc. milk
5	10:00 a.m.	Condition good. No tetany	5.0 grams in 200 cc. milk
6	9:00 a.m.	Tremors. Spasticity	5.0 grams in 200 cc. milk
7	7:30 a.m.	Severe tetany	5.0 grams in 200 cc. milk
	4:00 p.m.	Tetanic convulsion produced when tube touched larynx. Respiration stopped. Revived	
	9:00 p.m.	Tetanic convulsion aroused as before	
	9:20 p.m.	Dog limp and flaccid	5.0 grams in 200 cc. milk
8	8:30 a.m.	Tetanic convulsion aroused as above	
	9:00 a.m.	Dog limp and flaccid	10.0 grams in 200 cc. milk
	4:00 p.m.	No tetany	8.0 grams in 200 cc. milk
9	9:30 a.m.	No tetany	8.0 grams in 200 cc. milk
	4:00 p.m.	No tetany	10.0 grams in 200 cc. milk

DATE 1925	TIME	REMARKS	AMOUNT OF CALCIUM LACTATE GIVEN ORALLY BY STOMACH TUBE
April			
10	9:00 a.m.	No tetany	10.0 grams in 200 cc. milk
	4:00 p.m.	No tetany	10.0 grams in 200 cc. milk
11–14		No tetany	20.0 grams in 400 cc. milk daily
15	9:30 a.m.	No tetany. Diarrhea. Hair falling away in patches	5.0 grams in 200 cc. milk
	4:00 p.m.	No tetany. Coughing	5.0 grams in 200 cc. milk
16–17		No tetany. Coughing. Weak	10.0 grams in 400 cc. milk daily
18	8:00 a.m.	Ataxic. Spastic. Weak and cannot walk. Coughing worse	10.0 grams in 200 cc. milk daily
	2:45 p.m.	Spasticity relieved	10.0 grams in 200 cc. milk
19	7:30 a.m.	Condition improved. No tetany	10.0 grams in 200 cc. milk
	4:30 p.m.	No tetany. Severe diarrhea	10.0 grams in 200 cc. milk
20	10:00 a.m.	Slight spasticity. Coughing	6.0 grams in 200 cc. milk
	1:00 p.m.	No tetany. Very weak	3.0 grams in 200 cc. milk
	4:00 p.m.	No tetany. Cannot stand	6.0 grams in 200 cc. milk
21	8:00 a.m.	No tetany. Very weak. Coughing	
	10:00 a.m.	Died in depression. At autopsy the lower and middle lobes of both lungs were found to be entirely consolidated and the pleura hemorrhagic. The primary cause of death was pneumonia. No accessory parathyroid tissue was found	

Milk in the treatment of parathyroid tetany. The importance of diet in determining the severity of the syndrome following complete thyro-parathyroidectomy in dogs has been recognized by some investigators for many years. In general it has been observed that dogs on an exclusive meat diet develop tetany and die much sooner than dogs that are starved or are given meat-free diets. Dragstedt and Peacock (1923) in particular have emphasized the significance of diet and have demonstrated that it is possible to keep adult dogs alive and in good condition after complete thyro-parathyroidectomy by adding to the diet from 50 to 125 grams of lactose per day. This observation has been confirmed by Inouye (1924) who has demonstrated in addition that similar results may be obtained by feeding galactose. Glucose sucrose and dextrin however were found to be ineffective.

Dragstedt and Peacock (1923) found that a diet of white bread, skim milk ad libitum (300 to 400 cc. per day), and lactose was particularly effective in preventing the appearance of parathyroid tetany and in pre-

serving the life of completely parathyroidectomized dogs. At the time this work was done it was not known that such animals could be kept alive and free from tetany if given large doses of calcium lactate daily by mouth. Many observers have noted the beneficial effects of a milk diet in parathyroid tetany and some have attributed this effect to its content of calcium (Salvesen 1923). Greenwald (1924) has suggested that the results obtained by Dragstedt and Peacock (1923) were due to the calcium in their diets, presumably to that in their milk. It has thus appeared important to determine if milk alone can conserve the life of completely thyro-parathyroidectomized dogs. In this connection it should be stated that Dragstedt and Peacock (1923) were able to preserve the life of completely thyro-parathyroidectomized dogs by diets containing lactose but no milk. This has also been confirmed by Inouye (1924).

In the following experiments a complete thyro-parathyroidectomy was done in four adult female dogs. They were fed white bread and water during the course of the experiment, a diet which should make conditions most favorable for the control of tetany.

Experimental results. Dog 83. Adult female. Weight, 8 kgm.

On February 25, 1925, a complete thyro-parathyroidectomy was done. Animal was given from 400 to 500 cc. of skim milk daily by means of a stomach tube but in spite of this developed severe tetany within 72 hours. This could be controlled by the oral administration of 20 grams of calcium lactate daily, but when this was discontinued a fatal tetany ensued. At autopsy no accessary parathyroid tissue was found.

Dog 84. Adult female. Weight, 8 kgm.

On February 25, 1925 a complete thyro-parathyroidectomy was done. The administration of 400 cc. of milk daily did not prevent tetany, but subsequently 1000 cc. daily kept the animal free from tetany for two weeks. This animal eventually died in tetany. At autopsy no accessary parathyroid tissue was found.

Dog 90. Adult female. Weight, 8 kgm.

On March 24, 1925, a complete thyro-parathyroidectomy was done. This animal was given 500 cc. of milk by stomach tube on the day of operation, 1000 cc. on the day following, yet developed an unusually severe form of tetany on the third day, which required 20 grams of calcium lactate given by stomach tube, to control. From March 27 to 30, 1500 cc. of milk were given daily but the animal developed severe tetany each day and required 20 to 30 grams of calcium lactate for relief. On all these occasions of tetany unusual convulsive attacks were observed. When the observer approached the cage the animal gave a loud cry and immediately fell on its side in a violent tonic convulsion. The respiration stopped and the tonic spasm was followed by a period of complete flaccidity, during which the animal did not breathe and appeared moribund. With artificial respiration it was shortly restored to a nearly normal state. On March 31 the quantity of milk was increased to 2300 cc. Severe tetany again occurred and 30 grams of calcium lactate were required to save the animal's life. On April 1, 1850 cc. of milk were given and the animal remained free from tetany during the day but died in tetany on the succeeding day. No accessory parathyroid tissue was found at autopsy.

Dog 91 shows definitely that milk given in amounts of from 1500 cc. to 2900 cc. daily will not prevent the tetany of pregnancy and lactation. The protocol of this animal is given in detail.

Dog 91. Adult female. Weight, 10 kgm. Diet: bread and water. Pregnant.

DATE 1925	TIME	REMARKS	AMOUNT OF SKIM MILK GIVEN ORALLY BY STOMACH TUBE	ACCESSORY TREATMENT
			cc.	
March				
24	3:00 p.m.	Complete thyro-parathyroidectomy	500	
25	8:00 a.m.	Severe tetany		20 grams calcium lactate given by stomach tube
	1:00 p.m.	No tetany	500	
	4:30 p.m.	No tetany	500	
26	8:00 a.m.	Severe tetany		20 grams calcium lactate given by stomach tube
	9:45 a.m.	Severe tetany		30 grams calcium lactate given by stomach tube
	10:15 a.m.	Severe tetany		50 cc. of 7 per cent calcium chloride given intravenously
	1:30 p.m.	No tetany	500	
	4:30 p.m.	No tetany	500	
27	9:00 a.m.	Severe tetany		30 grams calcium lactate given by stomach tube
	10:00 a.m.	Slight tetany	500	
	3:00 p.m.	No tetany	500	
	4:30 p.m.	No tetany	500	
28	10:00 a.m.	No tetany	500	
	12:30 p.m.	Moderate tetany		30 grams calcium lactate given by stomach tube
	3:30 p.m.	Slight tetany	500	
29	9:30 a.m.	Moderate tetany	1000	30 grams calcium lactate given by stomach tube
	5:00 p.m.	No tetany	350	
30	9:30 a.m.	No tetany	500	
	3:00 p.m.	No tetany	1000	
31	8:30 a.m.	Severe tetany		30 grams calcium lactate given by stomach tube
	12:30 p.m.	No tetany	1000	
	4:30 p.m.	No tetany	500	
April				
1	10:00 a.m.	No tetany	1000	
	12:30 p.m.	Moderate tetany		30 grams calcium lactate given by stomach tube
2	9:00 a.m.	No tetany	1000	
	1:00 p.m.	No tetany	1000	
	4:00 p.m.	No tetany	900	
3	8:30 p.m.	Moderate tetany		30 grams calcium lactate given by stomach tube
	10:30 a.m.	No tetany	1000	
	4:00 p.m.	No tetany	1000	

DATE 1925	TIME	REMARKS	AMOUNT OF SKIM MILK GIVEN ORALLY BY STOMACH TUBE	ACCESSORY TREATMENT
			cc.	
April				
4	9:30 a.m.	Severe tetany	500	30 grams calcium lactate given by stomach tube
	11:30 a.m.	No tetany	1000	
5	10:00 a.m.	Moderate tetany	1000	30 grams calcium lactate given by stomach tube
6	11:00 a.m.	No tetany	1000	
	1:30 p.m.	No tetany	1000	
7	12:30 p.m.	No tetany	1000	
	4:00 p.m.	No tetany	500	35 grams calcium lactate given by stomach tube
8	9:00 a.m.	No tetany	1000	
	4:00 p.m.	No tetany	1000	
9	9:30 a.m.	Severe tetany		30 grams calcium lactate given by stomach tube
	10:30 a.m.	Severe tetany		50 cc. of 7 per cent calcium chloride given intravenously
10	8:00 a.m.	No tetany		
	9:00 a.m.	No tetany. Delivered 2 dead fetuses		
	4:00 p.m.	No tetany	500	30 grams calcium lactate given by stomach tube
11	8:00 a.m.	No tetany. Delivered one living pup and 2 dead		
	11:00 a.m.	No tetany	500	30 grams calcium lactate given by stomach tube
12–20		Slight tetany daily	500 (daily)	30 grams calcium lactate daily
21		Lactating		
21–24		Moderate tetany. Lactating	500 (daily)	40 grams calcium lactate
April 25 –May 29		No tetany. Lactating	500 (daily)	40 grams calcium lactate daily
May 30–June 3		No tetany	1000 (daily)	No calcium lactate
4		Severe tetany	1000 (daily)	40 grams calcium lactate
5		Used for other experiments		

In the report by Dragstedt and Peacock (1923) the dogs that were given "white bread and skimmed milk ad libitum, and lactose (50 to 125 grams

per day)" usually drank from 200 cc. to 400 cc. of milk daily and in no case received more than 500 cc. daily. On the basis of these facts it seems logical to conclude that it is not the milk in this diet that alone accounts for its effectiveness in preventing tetany. From the following table illustrating in part the composition of milk, it will be seen that cow's milk contains only 0.1687 gram of calcium as calcium oxide per 100 cc. Thus in 500 cc. or the maximum amount of milk given to any of these animals there was present only 0.8435 gram of calcium oxide or an amount which in our experience is far too small to have any effect whatever in tetany when given by mouth. On the other hand lactose is present in milk in more significant quantities. When milk is given in amounts of from 1000 cc. to 1500 cc. daily as was done in the experiments reported in this paper, the dog receives from 1.687 to 2.53 grams of calcium oxide and 47.5 to 71.25 grams of lactose per day. It is quite evident from the experiments of Dragstedt and Peacock (1923) and from those reported by Luckhardt and Goldberg (1923), Compere and Luckhardt (1924), as well as those reported in this communication that the ameliorating effect of a milk diet in tetany is far more likely to be due to its content of lactose than its content of calcium.

TABLE 1

SUBSTANCE	COW'S MILK	HUMAN MILK
	grams	*grams*
CaO	0.1687	0.0458
P_2O_5	0.2027	0.0345
Lactose	4.75	7.50

Modified from Holt, *Diseases of Infancy and Childhood*, showing the amount of certain constituents in 100 cc. of milk.

It has been frequently noted that a diet of cow's milk is more favorable for the development of infantile tetany than one of human milk. Cow's milk contains nearly four times as much calcium as is present in human milk so it does not appear that this factor is of major importance. Human milk on the other hand contains considerably more lactose and much less phosphate than cow's milk. Phosphates given by mouth favor the development of tetany. It seems quite probable therefore that the superiority of human milk over cow's milk in preventing tetany is due rather to its greater amount of lactose and decreased amount of phosphate than to its calcium content.

SUMMARY

1. Adult dogs may be kept alive and in good condition after a complete thyro-parathyroidectomy by the oral administration of calcium lactate. It required from 1.8 grams to 4.4 grams of calcium lactate per kilo of body

weight per day to keep these animals free from tetany. After from 5 to 11 weeks these animals remained in good condition and free from tetany without receiving any calcium medication. The work of Luckhardt and Goldberg (1923) is confirmed.

2. In a young dog, 6 weeks old, it required from 6 to 12 grams of calcium lactate per kilo of body weight per day given by mouth, to preserve its life following a complete thyro-parathyroidectomy and to control the post-operative tetany.

3. Pregnant dogs may likewise be kept alive and free from tetany following thyro-parathyroidectomy by the oral administration of calcium lactate. During the latter part of pregnancy and immediately preceding delivery it requires far more calcium to prevent tetany than at earlier periods.

4. Milk in amount of from 500 cc. to 1500 cc. daily did not prevent the appearance of parathyroid tetany in adult dogs nor preserve the life of these animals after thyro-parathyroidectomy. It was even less effective in a young dog. Milk given in amounts of from 1500 cc. to 2900 cc. daily did not control the tetany of pregnancy or lactation.

5. The suggestion is made that the well known ameliorating effect of a diet of cow's milk in parathyroid tetany is more probably due to its content of lactose than to its content of calcium. It is also suggested that the superiority of human milk over cow's milk in preventing infantile tetany is due to its greater amount of lactose and in part due to its decreased amount of phosphate rather than to its calcium content.

BIBLIOGRAPHY

COMPERE, E. L. AND A. B. LUCKHARDT. 1924. Proc. Soc. Exper. Biol. and Med., xxi, 526.

DRAGSTEDT, L. R. AND S. C. PEACOCK. 1923. This Journal, lxiv, 424.

DRAGSTEDT, L. R., K. PHILLIPS AND A. C. SUDAN. 1923. This Journal, lxv, 503.

DRAGSTEDT, L. R., A. C. SUDAN, AND K. PHILLIPS. 1924. This Journal, lxix, 477.

GREENWALD, I. 1924. Journ. Biol. Chem., lxi, 33.

INOUYE, T. 1924. This Journal, lxx, 524.

LUCKHARDT, A. B. AND B. GOLDBERG. 1923. Journ. Amer. Med. Assoc., lxxx, 79.

SALVESEN, H. A. 1923. Acta Med. Scand., Supplementum vi.

STUDIES ON THE PATHOGENESIS OF TETANY

VI. The Prevention and Control of Parathyroid Tetany by Strontium

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Berkeley and Beebe (1909) attempted to relieve the symptoms of tetany following thyro-parathyroidectomy in dogs by the injection of 2, 5 and 10 per cent solutions of strontium chloride. They state, "The effect of strontium salts has been tried upon ten animals in tetany, and it is scarcely possible to decide whether calcium salts give better results. The effects of the calcium appear a little earlier than those of the strontium, but they are not more complete or lasting." This statement refers to the temporary ameliorating effect of injections of calcium solutions in experimental tetany since at this time it was not believed that life could be indefinitely prolonged after parathyroidectomy by any type of calcium medication. In this connection it should be stated that measures that temporarily relieve parathyroid tetany may have quite a different significance from those that when daily applied repeatedly restore the animal to a normal state and conserve his life. Thus we have found many times that a dog in severe tetany may be entirely relieved for perhaps 24 hours simply by the introduction of hot water into the stomach. However this measure will not preserve his life although repeatedly applied. In the same way it has been observed that asphyxia may relieve all the symptoms of tetany for a long period. As possibly a new feature we have uniformly observed a severe spasm of the entire esophagus and in particular of the cardiac sphincter in parathyroid tetany in dogs. This is made evident by attempts to pass the stomach tube when the animal is beginning to show signs of an impending attack. Following a severe tonic convulsion during which the respiration stops, the esophagus will become completely relaxed. It may be significant that hyperpnea is such a prominent feature early in an attack of tetany and that asphyxia may in part relieve it.

Following the discovery by Luckhardt and Goldberg (1923) that parathyroid tetany in dogs could be controlled and that life could be greatly prolonged after a complete thyro-parathyroidectomy by the oral administration of calcium lactate, it seemed advisable to determine if the same

results might not be obtained with strontium lactate. Such evidence should help to decide the question of the specificity of the action of calcium in tetany since it does not appear probable that strontium can replace calcium in physiological processes. A complete thyro-parathyroidectomy was done in 8 adult dogs. They were fed white bread, corn meal and water throughout the duration of the experiment. They were given various amounts of strontium lactate daily either in solution or incorporated in corn meal, by means of a syringe and stomach tube. When severe tetany appeared and the life of the animal seemed threatened or when the strontium lactate was repatedly vomited, resort was made to the intravenous injection of a balanced solution of the following formula.

"Strontium Ringer's solution"

Sodium chloride	9.00 grams
Potassium chloride	0.42 gram
Strontium chloride	0.24 gram
Sodium bicarbonate	0.20 gram
Water q. s. ad	1000.00 cc.

The results of the experiment are given below.

Dog 59. Adult male. Weight, 10 kgm.

On February 21, 1924, a complete thyro-parathyroidectomy was done. Fifteen grams of strontium lactate were given daily by stomach tube and this animal remained free from tetany until February 29 when severe tetany was noted. Eight hundred cubic centimeters of "strontium Ringer's" solution given intravenously relieved this attack, 15 grams of strontium lactate were then given by stomach tube. The animal remained free from tetany for seven hours when it died in depression.

Dog 60. Adult female. Weight, 8 kgm.

On February 21, 1924, a complete thyro-parathyroidectomy was done. Fifteen grams of strontium lactate were given daily and animal remained free from tetany until March 10, when strontium medication was stopped for three days. The animal developed severe tetany on March 13 when 15 grams of strontium lactate were given orally. One hour after this treatment, when tetany usually subsided but in this case remained severe, 600 cc. of "strontium Ringer's" solution were given and the attack subsided. March 14 and 15 the animal remained free from tetany on 10 and 15 grams daily on these respective dates. March 16, dog was accidentally killed when stomach tube was passed into trachea. No accessary parathyroid tissue was found at autopsy. The gastric mucosa was hemorrhagic and heavily coated with tenacious mucus

Dog 64. Adult female. Weight, 9 kgm.

On March 11, 1924, a complete thyro-parathyroidectomy was done. Fifteen grams of strontium lactate were given daily by stomach tube and no tetany was noted until March 15, when tremors in the shoulder and temporal muscles appeared. These subsided after the strontium medication. March 16 tremors again appeared but subsided after 15 grams of strontium lactate were given. March 17 and 18 animal vomited but no tetany occurred. Fifteen grams of strontium lactate were given daily. Because of the vomiting which former experiments indicated was caused by the irritating properties of the strontium lactate on the gastric and intestinal mucosa, the daily dose of strontium lactate was reduced to 5 grams. On March 19 spasticity

was noted in the animal but this condition subsided after the strontium medication. March 20 no tetany or symptoms were apparent at 9 a.m. but the dog had developed distemper. At 1 p.m. tremors developed and at 4 p.m. severe tetany. Twelve hundred cubic centimeters of "strontium Ringer's" solution were given intravenously. At 4:20 p.m. all symptoms and tetany had subsided. March 21, the animal remained free from tetany, but the distemper was much worse. Five grams of strontium lactate were given orally. March 22, the animal was markedly depressed and died at 3 p.m. An extensive broncho-pneumonia was found at autopsy. No accessary parathyroid tissue was found.

Dog. 65. Adult female. Weight, 10 kgm.

On March 19, 1924, a complete thyro-parathyroidectomy was done. The animal remained free from tetany up to March 23 inclusive. Fifteen grams of strontium lactate were given daily by stomach tube. March 24, severe tetany appeared which subsided after 1200 cc. of "strontium Ringer's" solution were given intravenously. No tetany occurred on the following day but on March 26 severe tetany set in at 8 a.m. 10 grams of strontium lactate were given orally, but the animal died at 9:20 in depression, after all symptoms of tetany had subsided.

Dog 75. Adult male. Weight, 11 kgm.

On May 12, 1924, a complete thyro-parathyroidectomy was done. May 13, 14 and 15, tremors were noted which subsided after a daily dose of 20 grams of strontium lactate. May 16, the dog was depressed and constipated. Twenty grams of calcium lactate were given as substitute treatment and the strontium lactate omitted. From May 16 to 28 no tetany was noted and animal was apparently normal, except at the latter date when blood and mucus appeared in the stools. Twenty grams of strontium lactate had been given daily during this period. May 29 at 8:30 a.m. severe tetany, ataxia and muscular incoördination developed. Thirty grams of calcium lactate were given and at 10:30 a.m. tetany was relieved. At 11:30 animal became very weak and unable to stand. This condition progressed and the dog died at 1:30 p.m. in severe depression. At autopsy severe and extensive hemorrhagic gastro-enteritis was found. Blood in the large veins and heart remained unclotted two hours after death and remained unclotted in beakers at room temperature. No accessary parathyroid tissue was found.

Dog 82. Adult female. Weight, 14.5 kgm.

On February 11, 1925, a complete thyro-parathyroidectomy was done. Ten grams of strontium lactate were given daily by stomach tube and the dog remained free from tetany until February 15 at 7:30 p.m., when moderate tetany developed. Twenty grams of calcium lactate relieved this condition. February 16, no tetany was noted and 15 grams of strontium lactate and 50 cc. of liquid petrolatum (to correct constipation) were given. February 17, tremors were completely relieved by 10 grams of strontium lactate. February 18, animal first became spastic then vomited, and later developed severe tetany. Ten grams of calcium lactate obtained complete relief. February 19, severe tetany again developed and 150 cc. of 1.5 per cent calcium chloride solution by vein and 10 grams of calcium lactate by mouth were given as accessary treatment. February 20 to 26 inclusive the animal remained free from tetany. Fifty grams of kaolin and 50 cc. of liquid petrolatum were given daily. February 27, severe tetany developed and 20 grams of calcium lactate were given. February 28 to March 4, 9 a.m., the dog remained free from tetany, when severe tetany developed which was fatal before treatment could be given. Fifty grams of kaolin and 50 cc. liquid petrolatum were given daily during this interval. An extensive hemorrhagic gastro-enteritis was found at autopsy. No accessary parathyroid tissue was found.

The protocols of dogs 65 A and 61 are given in detail.

Dog 65 A. Adult male. Weight, 14.5 kgm.

DATE 1924	TIME	REMARKS	AMOUNT STRONTIUM LACTATE GIVEN ORALLY BY TUBE	ACCESSARY TREATMENT
March				
21	11:00 a.m.	Complete thyro-parathyroidectomy		
22–23		Depressed	10 grams daily	
24	10:00 a.m.	Depressed		1300 cc. strontium Ringer's solution given intravenously
	8:00 p.m.	Condition good		1200 cc. strontium Ringer's solution given intravenously
25	10:00 a.m.	Violent tetany		1200 cc. strontium Ringer's solution given intravenously
	10:20 a.m.	Tetany relieved		
25	9:00 p.m.	Severe tetany		1200 cc. strontium Ringer's solution given intravenously
	9:30 p.m.	Tetany relieved		
26	9:00 a.m.	Severe tetany		1200 cc. strontium Ringer's solution given intravenously
	9:20 a.m.	Tetany relieved		
	2:30 p.m.	Severe tetany		1200 cc. strontium Ringer's solution given intravenously
	3:00 a.m.	Tetany relieved		
	7:30 p.m.	Severe tetany		1100 cc. strontium Ringer's solution given intravenously
	7:50 p.m.	Tetany relieved		
27	9:00 a.m.	Severe tetany		1000 cc. strontium Ringer's solution given intravenously
	9:15 a.m.	Tetany relieved		
	3:00 p.m.	Moderate tetany		1100 cc. strontium Ringer's solution given intravenously
	3:15 p.m.	Tetany relieved		
	7:30 p.m.	Moderate tetany		1200 cc. strontium Ringer's solution given intravenously
	8:00 p.m.	Tetany relieved		

DATE 1924	TIME	REMARKS	AMOUNT STRONTIUM LACTATE GIVEN ORALLY BY TUBE	ACCESSARY TREATMENT
March				
28	9:30 a.m.	Severe tetany		1200 cc. strontium Ringer's solution given intravenously
	10:00 a.m.	Tetany relieved		
	2:00 p.m.	Marked ataxia		1100 cc. strontium Ringer's solution given intravenously
28	8:00 p.m.	Marked ataxia Weakness		1200 cc. strontium Ringer's solution given intravenously
29	10:00 a.m.	Died in tetany. No accessory parathyroid tissue found at autopsy. A severe gastro-enteritis was present		

Dog 61. Adult male. Weight, 14 kgm. Diet: bread, corn meal mush, water

DATE 1924	TIME	REMARKS	AMOUNT STRONTIUM LACTATE GIVEN ORALLY BY TUBE	AMOUNT OF STRONTIUM RINGER'S SOLUTION GIVEN INTRAVENOUSLY
				cc.
March				
6	9:00 a.m.	Complete thyroparathyroidectomy		
7–9		No tetany	15 grams daily	
10	3:00 p.m.	Severe tetany	15 grams	
	5:00 p.m.	Tetany relieved		
11	9:00 a.m.	Severe tetany		1200
	9:30 a.m.	Tetany relieved		
	3:00 p.m.	No tetany	15 grams	
12	9:00 a.m.	Moderate tetany	15 grams	
	2:00 p.m.	Severe tetany		1000
	2:30 p.m.	Tetany relieved		
13	9:00 a.m.	Severe tetany		800
	11:00 a.m.	Tetany relieved		
	3:00 p.m.	Moderate tetany		1200
	4:00 p.m.	Tetany relieved		
	10:00 p.m.	Moderate tetany		1200
	10:30 p.m.	Tetany relieved		
14	10:15 a.m.	Severe tetany		1200
	10:45 a.m.	Tetany relieved		
	4:00 p.m.	Slight tetany		1200
	4:30 p.m.	Tetany relieved		
	10:00 p.m.	Uncomfortable, no tetany		1200
15	9:00 a.m.	Slight tetany		1200
	10:00 a.m.	No tetany, vomiting		
	4:00 p.m.	Groaning		1200

DATE 1924	TIME	REMARKS	AMOUNT STRONTIUM LACTATE GIVEN ORALLY BY TUBE	AMOUNT OF STRONTIUM RINGER'S SOLUTION GIVEN INTRAVENOUSLY
				cc.
March				
16	10:30 a.m.	Tremors, groaning		1200
	11:00 a.m.	No tetany, vomiting		
	10:30 p.m.	Tremors, groaning		1200
	10:45 p.m.	More comfortable		
17	10:00 a.m.	Groaning, temperature 104		1200
	10:15 a.m.	More comfortable		
	10:00 p.m.	Tremors, groaning		1200
	10:10 p.m.	Tremors relieved		
18	9:30 a.m.	Severe tetany		1200
	9:40 a.m.	Tetany relieved		
	10:00 p.m.	No tetany		1200
19	9:00 a.m.	No tetany		1200
	9:00 p.m.	Groaning, vomiting		1200
20	9:00 a.m.	No tetany, vomiting		1200
	4:00 p.m.	No tetany, groaning		1200
21	9:30 a.m.	Severe tetany		1200
	9:40 a.m.	Tetany relieved		
	3:00 p.m.	No tetany, groaning		1200
	10:00 p.m.	No tetany, groaning		1200
22	9:00 a.m.	No tetany, vomiting		1200
	9:15 a.m.	More comfortable		
	8:00 p.m.	No tetany, vomiting		1200
23	11:00 a.m.	No tetany, groaning		1200
	7:00 p.m.	No tetany, vomiting		1200
24	9:00 a.m.	Tremors		1200
24	1:00 p.m.	Severe tetany		1200
	1:10 p.m.	No relief, convulsions becoming progressively more severe resulted in death at 1:15 p.m. No accessory parathyroid tissue found at autopsy. An extensive hemorrhagic gastro-enteritis was present		

It may be noted that dogs 59 and 65 were treated chiefly with strontium lactate by mouth. They lived 8 days and 7 days respectively, both dying in depression. Dogs 60 and 64 were likewise treated with strontium lactate orally, one being killed accidentally on the 24th day and the other dying of pneumonia on the 11th day. Dogs 75 and 82 were treated with strontium lactate but several times calcium lactate had to be given as accessary treatment. These animals lived 17 and 21 days, respectively. The experiments illustrate that strontium lactate when given by mouth has a very definite effect in relieving parathyroid tetany and in prolonging the life of thyro-parathyroidectomized dogs. It is not however so effective as calcium lactate. Strontium lactate is very irritant and invariably

produces and extensive catarrhal and hemorrhagic gastro-enteritis and this no doubt in part at least accounts for its less favorable effect. Luckhardt and Compere (1924) showed that irritant substances such as croton oil may induce severe tetany in animals that have long recovered from the immediate post-operative tetany. In dogs 61 and 65 A an attempt was made to control the tetany by the repeated intravenous injection of large amounts of a solution similar to Ringer's except that strontium chloride had been substituted in place of calcium chloride in the usual formula. These animals lived 18 and 9 days respectively. In dog 61, tetany appeared first on the 4th day and reappeared in severe form almost daily thereafter. This dog would most certainly have died in the first week without treatment.

SUMMARY

1. Parathyroid tetany in dogs may be relieved by the oral administration of strontium lactate in doses from 1.0 gram to 1.5 grams per kilo of body weight.

2. The life of adult dogs following a complete thyro-parathyroidectomy has been prolonged for at least 24 days by the oral administration of strontium lactate. It is more difficult to preserve the life of thyro-parathyroidectomized dogs by the oral administration of strontium lactate than it is by calcium lactate. This may be due to the very irritant effect which strontium lactate exerts on the gastro-intestinal mucous membrane.

3. Parathyroid tetany in adult dogs may be repeatedly relieved by the intravenous injection of large amounts of a modified Ringer's solution in which strontium chloride has been substituted in place of calcium chloride in the usual formula.

4. The life of adult dogs following a complete thyro-parathyroidectomy may be preserved for at least 18 days by the repeated daily injection of large amounts of this modified solution.

BIBLIOGRAPHY

BERKELEY, W. N. AND S. P. BEEBE. 1909. Journ. Med. Res., xx, 149.

LUCKHARDT, A. B. AND B. GOLDBERG. 1923. Journ. Amer. Med. Assoc., lxxx, 79.

LUCKHARDT, A. B. AND E. L. COMPERE. 1924. Proc. Soc. Exper. Biol. and Med., xxi, 523.

STUDIES ON THE PATHOGENESIS OF TETANY

VII. The Prevention and Control of Parathyroid Tetany by the Oral Administration of Kaolin

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In 1922 Dragstedt and in 1923 Dragstedt and Peacock reported that they were able to keep adult dogs alive and free from tetany after a complete thyro-parathyroidectomy by the addition of 50 to 125 grams of lactose daily to the diet of these animals. This amount of lactose was found to produce a very definite and characteristic change in the feces, which shortly became semi-fluid in consistency, light brown in color, acid to litmus, relatively odorless, and the fecal bacteria predominantly aciduric in type. Based upon this and certain other evidence reported in these papers it was suggested that parathyroid tetany was due for the most part to the absorption of toxic substances from the gastro-intestinal tract, which are not formed under the conditions obtaining when lactose is fed. The work of Kendall (1918), Torrey (1919), Hull and Rettger (1917), Cannon (1920), (1921), and others has shown very clearly that the type of intestinal bacteria and their metabolic activity is very greatly influenced by diet. A lactose diet such as the one Dragstedt and Peacock (1923) found effective in preventing parathyroid tetany in dogs, uniformly brings about the development of an intestinal flora that is predominantly aciduric in type and as emphasized by Kendall furnishes the intestinal organisms with a utilizable carbohydrate in the presence of which proteins are spared from bacterial cleavage. The work of Dragstedt and his associates (1917), (1919), (1920) indicates that the toxicity of the intestinal content is due for the most part to substances resulting from bacterial proteolysis. They found that the toxemia incident to acute intestinal obstruction was uniformly associated with the presence of a proteolytic intestinal flora and that furthermore this toxemia could be delayed in proportion to the degree to which bacterial proteolysis could be prevented. Lactose was found to be the most effective carbohydrate in checking this intestinal proteolysis or putrefaction and Inouye (1924) found it to be more effective than similar amounts of other carbohydrates in preventing parathyroid tetany.

It is without effect if given parenterally. It has been suggested that lactose prevents tetany through facilitating the absorption of calcium from the gastro-intestinal tract. It should be emphasized however that it requires very large doses of calcium lactate orally (from 20 to 50 grams daily in a 10 kilogram dog in our experience) to prevent tetany even with a meat-free diet. It appears improbable that lactose could bring about the absorption of so much calcium from a diet of bread and water or bread and 300 cc. of milk as is absorbed under the relatively huge calcium medication described.

Nevertheless there is no doubt that the fermentation of lactose yields acid radicals and there is some evidence that the absorption of calcium is facilitated by a change in the reaction of the intestinal content toward the acid side. The discovery by Braafladt (1923) that certain specific intestinal infections and intoxications could be controlled by the oral administration of kaolin, suggested its employment by us in parathyroid tetany and a brief statement of our preliminary findings appears in his paper. Braafladt (1923) showed that when kaolin is mixed with filtrates containing toxins or toxic products of Vib. cholerae, B. dysenteriae (Shiga) B. enteritidis, B. diphtheriae, B. botulinus, B. typhosus and B. paratyphosus, it combines with and removes these substances from the filtrate leaving it non toxic. The nature of this reaction between kaolin and the toxic products of bacterial growth is not definitely known but he suggests that it is probably an electrical one. Kaolin was also found to change the intestinal flora of rats, dogs and men from a predominantly proteolytic type to an aciduric one. In diseases caused by the proliferation of certain organisms in the intestines and the absorption of their toxic products, such as Asiatic cholera, bacillary dysentery, chronic ulcerative colitis, and acute enteritis, the oral administration of kaolin was found to be definitely advantageous and in some cases curative.

Kaolin contains approximately 47 per cent silica, 40 per cent aluminum oxide, and 13 per cent water. It is insoluble in water, cold dilute acids or alkalies. When given by mouth its effects would appear to be entirely local, acting through the absorption of products of bacterial growth, change in the type of the intestinal flora, or mechanically in protecting the intestinal mucosa. A complete thyro-parathyroidectomy was done in 10 adult dogs. They were fed white bread, corn meal and water during the experiment. From 50 to 200 grams of kaolin (Merck) suspended in water or cornmeal mush was given daily by means of a stomach tube and syringe. Under this medication the feces soon became white and chalk-like in character. Petrolatum was given in the later experiments to counteract the tendency of the kaolin to cause constipation. A careful examination of the following experiments indicates very clearly that kaolin when given orally to dogs is effective in controlling parathyroid

tetany and in preserving the life of completely thyro-parathyroidectomized dogs. We were in no case however able to preserve the life of these animals without occasionally resorting to additional measures such as the intravenous injection of Ringer's solution or the oral administration of calcium lactate. Kaolin is accordingly in our experience not so effective as either of these measures alone in controlling tetany. That it has a definite effect however is illustrated in the case of dog 80. This animal was kept free from tetany from February 4 until February 12 by the daily oral administration of 50 grams of kaolin and 50 cc. of liquid petrolatum. The kaolin was then withheld for 4 days and severe tetany ensued. This was controlled by 20 grams of calcium lactate. The subsequent employment of kaolin kept the animal free from tetany for 13 days. In dogs 85, 86 and 89 an attempt was made to secure the same results by the oral administration of wood charcoal. Although some slight effect may have been obtained it was not nearly so marked as in the case of kaolin. It is well known however that the adsorbent action of many substances has a definite specificity or selective character so that one adsorbent may take up certain substances whereas another adsorbent may not do so at least to so great an extent.

EXPERIMENTAL RESULTS. *Dog 50.* Adult female. Weight, 8 kgm.

On April 25, 1923, a complete thyro-parathyroidectomy was done. Two hundred grams of kaolin were given on the 26th and 27th but severe tetany set in at 6 a.m. on April 28 which required 1300 cc. of Ringer's solution to relieve. At 6 p.m. the same day severe tetany recurred and 15 grams of calcium lactate were given by stomach tube which produced complete relief from all symptoms. On April 29 tremors were noted and 15 grams of calcium lactate were required to relieve this condition. On April 30, 200 grams kaolin and 500 cc. milk were given and the animal remained free from tetany. May 1, 100 grams kaolin and 300 cc. milk were given and only slight tremors were noted. May 2 and 3, the same treatment was given and no tetany occurred. May 4, severe tetany occurred and besides 50 grams of kaolin, 15 grams of calcium lactate were given after which complete relief was obtained. May 5, 100 grams kaolin were given and no tetany occurred. May 6, severe tetany again set in which subsided after 100 grams kaolin and 15 grams calcium lactate were given. From May 7 to 16, 200 grams kaolin were given daily and the animal remained free from tetany. On May 17 moderate tetany set in and besides 100 grams of kaolin, 15 grams of calcium were required for relief. From May 18 to 25, 100 grams kaolin were given daily and no tetany occurred. May 26, tremors were noted and 15 grams calcium lactate were given as accessary treatment. The animal then remained free from tetany until June 3, when 15 grams of calcium lactate were necessary to relieve a severe attack of tetany. One hundred grams of kaolin were given daily in this interval. June 4 to 29, 300 grams of kaolin were given daily and the animal remained free from tetany. May 29, severe tonic and clonic convulsions occurred when the animal became excited and these attacks were completely relieved by warm water given by stomach tube. From June 30 to July 25, 200 grams kaolin were given daily and no tetany was manifested. Kaolin administration was then stopped and no tetany or symptoms were noted until November 20, when severe tetany occurred. Thirty grams of calcium lactate were required to relieve this attack. The dog then

remained free from tetany without medication until January 28, 1924, when severe and fatal tetany occurred. This attack was no doubt precipitated by oestrus. Autopsy revealed no accessary parathyroid tissue. The blood in the large vessels did not coagulate for several hours. Bilateral, well developed cataracts were present.

Dog 51. Adult female. Weight, 11 kgm.

A complete thyro-parathyroidectomy was done May 12, 1923. The following day 150 grams of kaolin and 600 cc. of milk were given. May 14 to 17 inclusive, 200 grams kaolin were given daily. May 18, the first signs of tetany occurred and 15 grams of calcium lactate were given as accessary treatment. May 19, the animal was given 200 grams kaolin, and a state of depression developed which ended in death the following day.

Dog 52. Adult female. Weight, 9 kgm.

July 6, 1923, at 3 p.m., a complete thyro-parathyroidectomy was done. With the exception of the first two days after operation, when 200 grams of kaolin were given daily, 100 grams of kaolin and 30 cc. of liquid petrolatum were given daily throughout the experiment. Tetany developed July 8, and 1400 cc. of Ringer's solution were given intravenously which obtained complete relief. Tremors and spasticity again developed July 11, when 1300 cc. Ringer's solution were given intravenously. Tetany again developed July 14 and was controlled by 1300 cc. Ringer's solution. July 15, 7 p.m., tetany developed and was controlled by 1000 cc. Ringer's solution. July 19, tetany developed. On this occasion the dog gave a sudden cry and fell on its side in a tonic convulsion. This stopped in a few minutes and was followed by complete flaccidity. The heart beat was slow and forcible. Respiration stopped for a time and then returned very gradually. The animal lay in a comatose state for five minutes, then quite suddenly regained its feet and appeared none the worse for the attack. Six convulsions of this nature occurred later in the evening. Nine hundred cubic centimeters Ringer's solution were given after the first attack. July 20 the animal appeared normal, but developed fatal tetany the following day.

Dog 78. Adult female. Weight, 10 kgm.

December 9, 1924, a complete thyro-parathyroidectomy was done. December 10, 200 grams of kaolin and 50 cc. of liquid petrolatum were given by stomach tube. On December 11 and 12, 100 grams of kaolin and 50 cc. liquid petrolatum were given. No tetany developed during this interval, but on December 13 tremors were noted and 30 grams of calcium lactate as accessary treatment were given. From this date to December 18 inclusive, 50 grams kaolin and 50 cc. liquid petrolatum were given daily and no tetany was noted. December 19, 9:30 a.m., spasticity, tremors and coarse jerkings of muscle groups set in which developed into severe tetany. Ten grams of calcium lactate relieved the attack. The following day tetany again set in and besides the daily dose of 50 grams of kaolin and 50 cc. of liquid petrolatum, 20 grams of calcium lactate were given. December 21 and 22 no tetany was observed and the usual dose of 50 grams kaolin and 50 cc. liquid petrolatum were given. December 23 to 26, 100 grams kaolin and 100 cc. liquid petrolatum were given daily and no tetany occurred. December 26 to January 5, 1925, 10 grams of calcium lactate were given daily as accessary treatment and no tetany set in. From January 6 to 13, 50 grams of kaolin and 25 cc. liquid petrolatum were given daily and no tetany was observed. Kaolin and liquid petrolatum medication was then stopped and the animal showed no signs of tetany until May 12, 1925, when a severe form of tetany set in which was relieved by 20 grams of calcium lactate. The animal was then used for another experiment.

Dog 80. Adult female. Weight, 15 kgm.

January 23, 1925, a complete thyro-parathyroidectomy was done. From this date to February 13, 50 grams kaolin and 50 cc. liquid petrolatum were given daily by stomach tube. The first attack of tetany occurred January 26 and 30 grams of calcium lactate were given as accessary treatment. Tremors and spasticity were then noted January 30, which were relieved by 20 grams calcium lactate accessary treatment. February 2, tremors were noted which subsided after 10 grams of calcium lactate were given. February 4, tremors again appeared and 30 grams of calcium lactate accessary treatment were given. February 13 to 17 inclusive, kaolin and petrolatum medication was stopped and on February 18 severe tetany occurred. Twenty grams of calcium lactate were required to produce relief. February 19, 50 grams kaolin and 50 cc. liquid petrolatum daily medication was again resumed and no tetany again occurred until March 5, when 20 grams of calcium lactate were given. March 21, kaolin medication was stopped and animal remained free from tetany until July 7, 1925, when the animal was used for other experiments.

Dog 81. Adult female. Weight, 9 kgm.

January 30, 1925, a complete thyro-parathyroidectomy was done. From this date to March 4, 1925, 50 grams of kaolin and 50 cc. liquid petrolatum were given daily by stomach tube. Tetany was first noted in this animal February 2, when 30 grams of calcium lactate accessory treatment was given in divided doses of 20 and 10 grams respectively with two hours' interval. All symptoms subsided and the animal remained free from further symptoms until February 4, when tremors appeared which required 10 grams calcium lactate to relieve. February 5 to 27, severe attacks of sneezing occurred daily but no tetany or tremors developed. No accessory treatment was given. February 27, hyperpnea developed and 20 grams of calcium lactate were given. One hour later severe tetany set in and respiration stopped while 150 cc. of a 2 per cent solution of calcium lactate were being given intravenously. Artificial respiration revived the animal after which tetany subsided. March 2, some spasticity was noted, but this subsided without accessary treatment. March 4, spasticity appeared, followed by severe tetany which ended fatally. No accessory parathyroid tissue was found at autopsy. The blood in the large veins and chambers of the heart was still unclotted eight hours after death.

Dog 85. Adult female. Weight, 9 kgm.

March 6, 1925, a complete thyro-parathyroidectomy was done. Thirty grams of wood charcoal and 50 cc. liquid petrolatum were given daily by stomach tube. The animal remained free from tetany until March 9, when a severe attack terminated fatally. At autopsy no accessary parathyroid tissue was found. The blood in the large veins and chambers of the heart was unclotted.

Dog 86. Adult female. Weight, 11.5 kgm.

March 6, 1925, a complete thyro-parathyroidectomy was done. From this date until March 20, 30 grams of wood charcoal and 50 cc. liquid petrolatum were given by stomach tube. Severe tetany developed March 12, 13, 17 and 19 respectively. On each of these days 20 grams of calcium lactate accessary treatment were given and complete relief obtained. On March 20 the animal was placed in a semi-air-tight chamber (2 feet by 4 inches cube) for one hour after moderate tetany had developed. There was no apparent effect on the attack. Sixty cubic centimeters of 7 per cent calcium chloride solution were then given intravenously and tetany subsided. March 21 to 30, 50 grams of kaolin and 50 cc. of liquid petrolatum were given daily by stomach tube. Tetany developed March 22, and 20 grams of calcium lactate were necessary to relieve the attack. Tetany again set in on March 24, 25 and 27. On these occasions 20 grams calcium lactate accessory treatment were given. March 31 to April 11, kaolin treatment was stopped and 30 grams of calcium lactate were given

daily. Under this treatment tetany developed daily. A number of subcutaneous abscesses developed on the fore legs April 5. The animal died in tetany April 11. Autopsy revealed four deep seated abscesses and communicating sinuses in shoulders and legs. No accessory parathyroid tissue was found.

Dog 88. Adult female. Weight, 13.6 kgm.

March 11, 1925, a complete thyro-parathyroidectomy was done. Thirty grams of wood charcoal and 50 cc. liquid petrolatum were given daily by stomach tube. Tetany developed March 13 at 7 a.m. and 20 grams of calcium lactate were given. Tetany persisted until 2 p.m.; 30 additional grams of wood charcoal were given at this time. March 15, 17 and 19, severe tetany again developed which required, on each occasion, 20 grams of calcium lactate to relieve. From March 20 to March 24, 50 grams of kaolin and 50 cc. of liquid petrolatum were given daily by stomach tube and the animal remained free from tetany until March 24, when severe and fatal tetany developed. Autopsy revealed a large abscess in the neck about one of the buried ligatures. No accessory parathyroid tissue was found.

Dog 89. Adult female. Weight, 12 kgm.

March 11, 1925, a complete thyro-parathyroidectomy was done. Thirty grams of wood charcoal and 50 cc. of liquid petrolatum were given daily by stomach tube. Tetany developed March 14, and 20 grams calcium lactate, accessory treatment, were given. Tetany again developed March 16, 18 and 20. On each of these dates 20 grams of calcium lactate were given and the attacks subsided. From March 21 to April 18, 50 grams of kaolin and 50 cc. liquid petrolatum were given daily. Severe tetany developed on the following dates under this treatment, March 22, 29 and April 9. In the intervals between these periods and following until April 19 the animal appeared absolutely normal. April 14, the kaolin treatment was stopped and severe tetany developed April 19. Thirty grams calcium lactate were given and complete relief was obtained. Following this period to July 20 no further attacks of tetany were noted and the animal was used for other experiments.

SUMMARY

The oral administration of kaolin in amounts of from 50 to 200 grams was found to be effective in controlling parathyroid tetany and in preserving the life of completely thyro-parathyroidectomized dogs. It is suggested that this effect of kaolin is due to the adsorption of toxic products of bacterial growth in the gastro-intestinal tract and to the predominance of an aciduric intestinal flora which it brings about. These experiments are interpreted to support the view that parathyroid tetany is an intoxication and that the responsible toxic substances in non-pregnant animals arise chiefly in the gastro-intestinal tract as a result of bacterial proteolysis.

BIBLIOGRAPHY

BRAAFLADT, L. H. 1923. Journ. Infect. Dis., xxxiii, no. 5.

CANNON, P. R. 1920. Journ. Infect. Dis., xxvii, 139.

1921. Journ. Infect. Dis., xxix, 369.

DRAGSTEDT, L. R. 1922. Journ. Amer. Med. Assoc., lxxix, 1593.

DRAGSTEDT, L. R. AND S. C. PEACOCK. 1923. This Journal, lxiv, 424.

DRAGSTEDT, L. R., J. J. MOORHEAD AND F. W. BURCKY. 1917. Journ. Exper. Med., xxv, 421.

DRAGSTEDT, L. R., C. A. DRAGSTEDT, J. T. MCCLINTOCK AND C. S. CHASE. 1919. Journ. Exper. Med., xxx, 109.

DRAGSTEDT, L. R., P. R. CANNON AND C. A. DRAGSTEDT. 1920. Journ. Infect. Dis., xxvii, 139.

HULL, T. G. AND L. F. RETTGER. 1917. Journ. Bacteriol., ii, 47.

INOUYE, T. 1924. This Journal, lxx, 524.

KENDALL, A. I. 1918. Amer. Journ. Med. Sci., clvi, 157.

TORREY, J. C. 1919. Journ. Med. Res., xxxix, 415.

STUDIES ON THE PATHOGENESIS OF TETANY

VIII. The Effect of Guanidine Intoxication on the Blood Calcium of Parathyroidectomized Dogs

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The experimental work of Koch, Paton and Findlay (1916), Burns and Sharpe (1916) has led these workers to conclude that parathyroid tetany is an intoxication due to the accumulation of guanidine in the body of the parathyroidectomized animal.

The evidence presented by these workers consists for the most part in the reported demonstration of an increased amount of these toxic bases in the blood and urine of parathyroidectomized dogs and of the tetanogenic effect of the administration of these substances to normal dogs and in a few cases to animals in latent tetany. Paton and Findlay (1916) concluded that the symptoms observed following parathyroidectomy were undistinguishable from those produced in normal animals by the administration of guanidine. Furthermore in these animals an increased electrical excitability of the peripheral motor nerves was regularly found.

Dragstedt, Phillips and Sudan (1923) reported that in their experience guanidine and methyl-guanidine intoxication in normal dogs was quite different from typical parathyroid tetany in these animals, an observation confirmed by Nicholas and Swingle (1924). However, doses of guanidine and methyl-guanidine, which were too small to produce any effect in normal dogs, were found to induce symptoms such as spasticity, tremors, salivation, enophthalmos, blepharospasm and toxic convulsions very similar if not identical to those of parathyroid tetany, in dogs that had been thyroparathyroidectomized several months before and had been kept alive by lactose treatment. Parathyroid tetany appears only in animals without parathyroid glands and it may not be possible to produce it in animals whose parathyroid glands are intact.

The work of MacCallum and Vogel (1913), Howland and Marriott (1918), Salvesen (1923) and Collip (1925) has shown that one of the changes that uniformly accompanied tetany immediately following complete parathyroidectomy is a decrease in the concentration of the blood

calcium. While it has not yet been proved that the symptoms of tetany are due to this decreased blood calcium, it is apparent that it is much a part of the parathyroid symptom-complex.

In order to determine whether there is a corresponding fall in the blood serum calcium concentration following guanidine hydrochloride intoxication, or tetany in dogs completely thyro-parathyroidectomized, the following experiment was done.

Two adult female dogs from which the thyroids and parathyroid glands had been removed some months previously, the tetany controlled by oral administration of calcium lactate or kaolin, were put on a diet of corn meal mush and water. Determinations of the calcium concentration in the blood serum were then made before and after the administration, subcutaneously, of doses of 0.05 gram guanidine hydrochloride per kilogram body weight.

RESULTS. The detailed protocols of the experiment are given in tabular form.

Dog 89. Weight, 12.2 kgm. Diet: bread, corn meal, water

DATE 1925	TIME	REMARKS	SERUM CALCIUM
			mgm. 100 cc.
March 11	3:30 p.m.	Complete thyro-parathyroidectomy	
12–20		Tetany controlled by charcoal and calcium lactate given orally	
March 21–April 20		Tetany controlled by kaolin	
April 21–June 23		No tetany: no treatment	
June 24		Condition good: no tetany	10.3
26		Condition good: no tetany	9.4
29		Condition good: no tetany	9.5
July 1		Condition good: no tetany	9.6
3		Condition good: no tetany	10.2
7	12:30 p.m.	Condition good: no tetany	9.4
	1:00 p.m.	0.61 gram of guanidine HCl given subcutaneously	
8	1:55 p.m.	Condition good: no tetany	9.4
	2:20 p.m.	0.61 gram of guanidine HCl given subcutaneously	
9	2:10 p.m.	Condition good: no tetany	9.8
	2:15 p.m.	0.61 gram of guanidine HCl given subcutaneously	
	3:00 p.m.	Hyperpnea: no tetany	
10	12:20 p.m.	No tetany	10.5
	12:25 p.m.	0.61 gram of guanidine HCl given subcutaneously	

DATE 1925	TIME	REMARKS	SERUM CALCIUM
			mgm. per 100 cc.
July			
11	1:45 p.m.	No tetany	10.7
	2:00 p.m.	0.61 gram of guanidine HCl given subcutaneously	
12	10:10 a.m.	Slight spasticity, tremors	10.6
	10:15 a.m.	0.61 gram of guanidine HCl given subcutaneously	
	10:45 a.m.	Tremors, spasticity	
13		Condition good: no tetany	
14	3:30 p.m.	Occasional tremors	9.5
	3:35 p.m.	0.61 gram of guanidine HCl given subcutaneously	
15	2:00 p.m.	Condition good: no tetany	9.5
	2:05 p.m.	0.61 gram of guanidine HCl given subcutaneously	
	3:30 p.m.	Retching, vomiting, tremors	
16	4:20 p.m.	Depressed, very weak. No tetany	10.0
17	8:00 a.m.	Found dead in cage. No accessory parathyroid tissue found at autopsy. There were many small hemorrhages in the duodenal mucosa.	

Dog 91. Weight, 11.0 kgm. Diet: bread, corn meal, water

March 24	3:00 p.m.	Complete thyro-parathyroidectomy	
March 25–June 5		Tetany controlled by oral administration of calcium lactate	
June 6–28		Condition good: no tetany, no treatment	
29		Condition good: no tetany, no treatment	5.0
July 1		Condition good: no tetany, no treatment	5.0
3		Condition good: no tetany, no treatment	5.9
7	12:35 p.m.	Condition good: no tetany, no treatment	4.5
	1:00 p.m.	0.55 gram guanidine HCl given subcutaneously	
8	2:00 p.m.	No tetany	4.8
	2:25 p.m.	0.55 gram guanidine HCl given subcutaneously	
	3:30 p.m.	Sneezing, retching, tremors	
9	2:25 p.m.	No tetany	4.8
	2:30 p.m.	0.25 gram guanidine HCl given subcutaneously	
	3:00 p.m.	Slight sneezing	
10	12:30 p.m.	No tetany	5.1
	12:35 p.m.	0.50 gram guanidine HCl given subcutaneously	
	1:00 p.m.	Retching, tremors, coughing	
11	2:00 p.m.	No tetany	
	2:05 p.m.	0.55 gram guanidine HCl given subcutaneously	
	2:30 p.m.	Coughing, sneezing, retching, tremors	

DATE 1925	TIME	REMARKS	SERUM CALCIUM
			mgm. per 100 cc.
July			
11	2:40 p.m.	Clonic convulsions	
	4:00 p.m.	Recovery nearly complete	
12	10:30 a.m.	Spastic	4.7
	10:35 a.m.	0.55 gram guanidine HCl given subcutaneously	
	11:30 a.m.	Hyperpnea, severe tremors, retching	
13		No tetany	
14	9:00 a.m.	Severe tonic and clonic convulsions	
	11:00 a.m.	Severe tonic and clonic convulsions	
	2:45 p.m.	Severe tonic and clonic convulsions	
	3:20 p.m.	Recovery nearly complete	4.5
15	2:20 p.m.	Condition good. No tetany	3.9
	2:25 p.m.	0.55 gram guanidine HCl given subcutaneously	
	2:45 p.m.	Hyperpnea, tremors, spasticity	
	3:00 p.m.	Lies sprawled out in cage, very weak	4.0
16	9:00 a.m.	Tonic and clonic convulsions	
	4:30 p.m.	No tetany	3.8
17		Depressed, severe diarrhea, very weak	
18	8:00 a.m.	Found dead in cage. No accessary parathyroid tissue found at autopsy	

It may be noted in the protocols of dogs 89 and 91, that at no time during the experiment was there a decrease in the calcium content of the blood serum greater than was the normal variation in these animals. At no time during the experiment was a typical attack of tetany noted. Toxic symptoms were manifested by tremors, spasticity of muscle groups, hyperpnea and retching. These symptoms with added weakness became more marked following injections as the experiment progressed.

Dog 91 on several occasions had severe tonic and clonic convulsions following the administration of guanidine hydrochloride with practically no change in the blood serum calcium concentration.

CONCLUSION

In thyro-parathyroidectomized dogs that have been kept free from tetany over a period of several months, there is no marked decrease in the blood serum calcium concentration, following immediate or prolonged intoxication produced by guanidine hydrochloride.

BIBLIOGRAPHY

BURNS, D. AND J. SHARPE. 1916. Quart. Journ. Exper. Physiol., x, 315.
COLLIP, J. B. 1925. Journ. Biol. Chem., lxiii, 395.
DRAGSTEDT, L. R., K. PHILLIPS AND A. C. SUDAN. 1923. This Journal, lxv.
HOWLAND, J. AND W. M. MARRIOTT. 1917–18. Quart. Journ. Med., xi, 289.

KOCH, W. F. 1912. Journ. Biol. Chem., xii, 313; xv, 43.
1915–16. Journ. Laby. Clin. Med., i, 299.
1918. Med. and Surg., ii, 9.
MACCALLUM, W. G. AND K. M. VOGEL. 1913. Journ. Exper. Med., xviii, 618.
NICHOLAS, J. S. AND W. W. SWINGLE. 1924. This Journal, lxix, 455.
PATON, D. AND L. FINDLAY. 1917. Quart. Journ. Physiol., x, 315.
SALVESEN, H. A. 1923. Journ. Biol. Chem., lxi, 443.

STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

XVII. A Lasting Preparation of the Denervated Heart for Detecting Internal Secretion, with Evidence for Accessory Accelerator Fibers from the Thoracic Sympathetic Chain

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In previous papers of this series attention has been called to the value of the denervated heart as an indicator of changes occurring in the organism (Cannon, 1919; Cannon and Rapport, 1921). Our conviction that this preparation may be helpful in solving a number of important problems is based on certain features of cardiac muscle which mark it as a structure representative of widespread and significant bodily elements. These features may be briefly mentioned.

The cross-banded arrangement of cardiac muscle, even in the nodal regions, its quick response to brief stimulation, its rapid contraction and relaxation, and the all-or-none character of its activity are typical of skeletal muscle fibers. Skeletal muscle, it is estimated, constitutes about 43 per cent of the body weight (Vierordt, 1906). Unlike fat, bone, tendon, blood, skin and much of the nervous system (i.e., the fiber tracts and the nerve trunks), muscle is the seat of large chemical changes. It is probable that even when the muscles are at rest they are responsible for at least half of the total metabolism. Some of the conditions which influence cardiac muscle may give hints as to occurrences in the great masses of cross-banded muscle connected with bones.

The rate of the heart is closely related to the rate of its own metabolism and of general bodily metabolism. In the heart-lung preparation subjected to variations of temperature—between 32° and 39°C.—"the rate of gaseous metabolism varies almost exactly as the pulse rate, the oxygen consumption and carbon dioxide production *per beat* being the same at both

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temperatures" (Evans, 1912). As has been shown by observations made during prolonged fasting (Benedict, 1915), prolonged restricted diet (Benedict, Miles, Roth and Smith, 1919), the standard basal state (Harris and Benedict, 1919), muscular effort (Smith, 1922), and diseases with altered metabolism (Sturgis and Tompkins, 1920; Minot and Means, 1924), there is a fairly close relationship between the rate of the heart and the rate of total metabolism. Since cardiac rate, cardiac metabolism and total metabolism are thus bound together in the intact organism, even though the nervous system be intermediary, such influences as affect the rate and metabolism of the isolated heart may be significant in relation to metabolic changes in other muscular structures.

The rate of the heart isolated from the central nervous system is influenced only by changes of temperature, and by chemical agents brought to it in the blood stream—secretions of ductless glands and possibly products of digestion or metabolism—acting on the pace maker (Cannon and Rapport, 1921). Rogoff has argued that the denervated heart cannot yield reliable information because stimulation of the adrenal medulla, the liver and the thyroid, and also injection of extracts of various tissues will all cause a faster beat (Rogoff, 1924). One might quite as justifiably argue that intestinal muscle, used by him to determine adrenal secretion, is unreliable because heating the medium that surrounds it, or applying an excess of carbon dioxide, causes it to relax, just as adrenin does. With both indicators what is permitted to act on them is the important matter. When we have excluded action of the liver and thyroid and have not injected extracts, and then have noted that reflex acceleration, for example, occurs, but that it does not occur after inactivation of chromophil organs, an inference that the acceleration might be due to the liver, thyroid or organ extracts would be absurd. The only inference warranted is that the acceleration was due to increased activity of chromophil organs.

The denervated heart, therefore, may be reasonably considered as a representative portion of muscular tissue, probably capable of yielding insight into responses of both the striated and the non-striated forms of that tissue. It is a continually and rhythmically active muscle, living isolated in the body fluids. Its rate is fixed by influences affecting a small portion of its structure, the sinus node. The changes in rate, however, are capable of revealing alterations in the bathing fluids, (the "milieu interne," to use Bernard's phrase), and, as accumulating evidence tends to prove, revealing such alterations as influence contractile structures in general and also in specific ways. In short, it acts like an isolated organ perfused with different fluids—only it undergoes an *internal perfusion*. Its rate of action can be readily recorded electrically or by placing a tambour against the chest. A valuable addition to our methods, therefore, would be a preparation of the denervated heart under surgical condi-

tions so that animals thereafter could be kept alive for a considerable period and in normal health, for study of conditions influencing the rate of beat.

It appears that the only investigator who has hitherto reported having made such a preparation for the study of cardiac function is Friedenthal (1902). He destroyed the middle and lower roots of the left vago-accessory nerves at the medulla, and severed the right vagus below the recurrent branch. Then he removed the lower cervical and upper thoracic ganglia of the sympathetic chain. One dog and several rabbits survived the operation and were observed. The main features emphasized in the report on the dog were that the resting pulse was little changed from the normal, that the cardiac nerves are not essential for existence (the animal lived 8 months after the isolation was completed), and that capacity for muscular work (i.e., running) was much reduced. Unfortunately Friedenthal did not record the pulse. His statement that the rate of the denervated heart can still be markedly influenced by changes of blood pressure (which is incorrect (see Cannon and Rapport, 1921)) might indicate that the rate was not steady. As will be shown later, the conditions governing the heart are much more complicated than he realized, and the only proof of isolation from the central nervous system is relative indifference to disturbing conditions after humoral factors have been excluded. Friedenthal should have studied carefully the heart rate under different circumstances. This he apparently did not do.

In our experiments we have made use of the cat.

The innervation of the heart in the cat. The scattered distribution of nerves near the heart renders practically impossible any satisfactory denervation of the organ by operating in that region. It is necessary to find a place where the relations are simpler. At first we thought we might open the pericardium and by cutting and applying phenol we might destroy the nerves as they course along the vessels which connect the base of the heart with the pericardial wall. Our attempts were only partially successful, however, and we therefore turned elsewhere. The other situation in which the relations are relatively simple is at the origin of the cardiac nerves—the vagus trunks and the stellate ganglia. The chief difficulty with any simple exclusion of vagal influences, as by section of the nerves in the neck, lies in the consequent serious disturbances of respiration and digestion. To avoid these troubles one recurrent laryngeal nerve must be preserved in order that the larynx may act properly and protectively, and one vagus trunk must pass to the abdomen in order that the digestive functions may continue normal.

Some preliminary experiments showed that it is possible to exclude the influence of the right vagus by cutting strands which reach out from it towards the heart near the mouth of the superior vena cava and the

azygos vein. Similar strands on the left side, however, are much more readily found and severed. Because of the complexity due to the presence of the azygos vein on the right side, therefore, we selected the more readily accessible left vagus trunk for service to the gastro-intestinal canal. Investigation proved that the left recurrent laryngeal must be cut; that made necessary the preservation of the right recurrent.

Careful tests in acute experiments and also in numerous surgical operations have shown that the *right vagus* gives off all its inhibitory cardiac branches below the origin of the recurrent laryngeal (see fig. 1). Boehm and Nussbaum (1875) describe a very fine strand leaving the right vagus above the loop of the recurrent laryngeal and, having an independent course till it joins filaments from the stellate ganglion, forms with them a "common cardiac nerve." This fine strand corresponds to the right depressor nerve, as described by Perman (1924). The right recurrent laryngeal also, as a rule, sends off a twig to the common cardiac nerve (Boehm and Nussbaum, 1875; Dogiel and Archangelsky, 1906). This twig we have identified, but there appears to be no evidence that it has a vagal effect. The only stimulation of it reported by Boehm and Nussbaum did not alter the heart rate. Notwithstanding this negative evidence we have invariably excluded from action any filament running caudad from the recurrent loop, as well as the filaments passing mediad from the vagus trunk above the heart, by excision of a considerable extent of the common cardiac nerve on the front of the trachea (see fig. 1). After these fine nerve threads and the vagus trunk between the recurrent branch and the heart have been removed, stimulation of the right vagus at the top of the thorax or at various levels in the neck, even as far cephalad as the jugular ganglion, has, in our experience, no effect on the heart. In older literature reference is made to a cardiac connection of the superior laryngeal nerve with the recurrent laryngeal through the anastomosis of Galen (see François-Franck, 1880); we have found no change of heart rate on peripheral excitation of that nerve. Indeed, in the cat, according to our tests, no branches which affect the heart rate are given off from the cervical portion of the right vagus; they arise near the heart.

Our acute experiments and also our observations on numerous cases at the time of surgical operation and at subsequent tests have shown that the cardiac branches leave the *left vagus* trunk near the heart. There is considerable variation, however, in the places of origin. In some cases all the cardiac branches arise close below the recurrent laryngeal branch and no twigs arise from the latter. In other cases important cardiac branches leave the vagus trunk much lower, near the left pulmonary vessels, or pass off from the recurrent laryngeal. Perman (1924) describes a branch which, leaving the left vagus below the hilus of the lung, unites with a similar branch from the right vagus, and from this union a twig passes forward to

the dorsal wall of the auricles. This arrangement, first described by Perman, we have been able to verify in several instances. No cardiac branches arise above the recurrent, as shown in numerous tests by first cutting the recurrent and all other vagal branches below it as far as the junction with the esophagus, then stimulating the left vagal trunk high in the thorax or in the neck, and also the jugular ganglion, and finding no change recorded in the heart rate. Since it is very difficult to cut the fine filaments which may grow out to the heart from the recurrent laryngeal as it turns cephalad under the aortic arch, we adopted the plan of cutting the left recurrent branch itself. Occasionally it runs a relatively long independent course and for that reason may be overlooked. We have definite proof of renewal of vagal connections as early as 68 days after they had been severed (see p. 350), though regeneration probably does not commonly occur so soon.

There has been general agreement that the immediate sympathetic innervation of the heart is derived from the stellate and inferior cervical ganglia. Boehm and Nussbaum (1875), Langley (1892), and Dogiel and Archangelsky (1906) describe only such relations in the cat. In Gaskell's monograph (1920), "The Involuntary Nervous System," the accelerators are described as proceeding from the above mentioned ganglia, and from ganglia on the subclavian ansa. Langley represented all the thoracic nerves from I to V or VI as sending accelerator fibers upward through the sympathetic chain to the stellate, but not as having more direct cardiac connections. And Tigerstedt in his monumental "Physiologie des Kreislaufes" (1921) sums up present knowledge on the basis of Langley's evidence that the relays are in the stellate and inferior cervical stations. According to Boehm and Nussbaum, three sets of fibers are given off from the *right stellate:* two or three prominent strands join the vagus trunk; others pass mediad on the dorsal side of the vagus to join the common cardiac nerve mentioned above; and another strand goes to the esophagus and the hilus of the lungs. Our observations confirm the existence of these sets of fibers. The right inferior cervical ganglion, stimulation of which causes a faster heart beat, gives off branches to the nearby vagus trunk (see fig. 1, and also November 7, 10 and 14, table 1). In one case only out of sixty, Boehm and Nussbaum report finding a connection with a cervical root such as to make the inferior cervical independent. According to Langley the lower cervical spinal nerves of the cat contain no accelerator fibers; those fibers originate in the upper thoracic nerves, chiefly the second and third. The ganglion loses its central connection, therefore, on extirpation of the right stellate, for the preganglionic fibers of the inferior cervical practically always pass through the stellate. In our experience the peripheral connections of the inferior cervical with the heart are destroyed by cutting the right vagus below the recurrent laryngeal

branch; for after that cut the acceleration from exciting the ganglion fails to occur. We have not been able to produce any effect on the heart rate by stimulating the superior cervical sympathetic ganglion, nor have we found by dissection any branches from it to the heart.

A large strand (sometimes two) reaches caudad from the *left stellate*; some twigs from it commonly join the left vagus, while the rest of the strand continues towards the heart. There is also a small fiber, attaching usually at the region of the inferior cervical ganglion, that runs between the in-

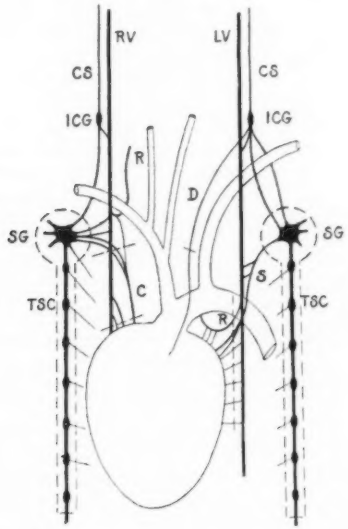

Fig. 1. Diagram of the usual arrangement of cardiac nerves in the cat. *R V*, right vagus; *L V*, left vagus; *C S*, cervical sympathetic; *I C G*, inferior cervical ganglion; *R*, recurrent laryngeal nerve; *D*, depressor nerve; *S G*, stellate ganglion; *C* "common cardiac nerve;" *S*, sympathetic fiber; *T S C*, thoracic sympathetic chain. See text for consideration of fibers reaching mediad from thoracic ganglia below the stellate. The dash lines indicate the parts cut or excised.

nominate and left subclavian arteries, and sends fibers to the ventral side of the aortic arch and onward towards the heart. Sometimes it runs free in the neck. It corresponds to the depressor nerve, as figured by Dogiel and Archangelsky. Although this fiber remained intact in our earlier operations it could not have mediated central or reflex accelerator effects, because removal of the stellate ganglion eliminated the preganglionic connections. When the cervical sympathetic trunk or the superior cervical ganglion was stimulated after the stellate had been removed, no change in

TABLE 1

Records of animals with stellate ganglia removed, vagus nerves cut in the neck, adrenal glands removed, liver nerves severed, and subjected to afferent brachial stimulation after being decorticated and recovering from ether. (Exceptions to these procedures are mentioned under "Remarks")

DATE (1924)	MAXIMUM REFLEX ACCELERATION OF HEART	REMARKS
	beats per minute	
October 31	20	Liver nerves not cut. No increase on stimulation of peripheral end right sciatic 30 seconds
November 3	30	Left adrenal in, left splanchnics cut. Maximum increase after removal of left adrenal, 5 beats
November 4	5	
November 5	4	
November 6	8	
November 7	0	Increase 28 beats on stimulation of right inferior cervical ganglion, but no increase after right vagus cut below recurrent branch. Increase 4 beats on stimulation of left inferior cervical ganglion
November 10	2	Increase on stimulation of inferior cervical ganglia; left, 14 beats, right, 22 beats
November 11	0	Vigorous abdominal massage over liver; increase 2 beats per minute
November 13	8	Left adrenal in, left splanchnics cut. Ether. No decerebration. Vigorous abdominal massage over liver; increase 4 beats
November 14	11	Left adrenal in, left splanchnics cut. Stimulation of right inferior cervical ganglion, increase 30 beats; no increase after vagotomy below recurrent branch
December 2, a.m.	20	Left adrenal in, left splanchnics cut 3 weeks previous. Right splanchnic stimulated, no increase
December 2, p.m.	16	Left adrenal in, left splanchnics cut 3 weeks previous
December 4, a.m.	10	Right and left splanchnics cut, liver nerves not cut, both adrenals in
December 4, p.m.	22	Right and left splanchnics cut, liver nerves not cut, both adrenals in. Ether
December 5	12	Right and left splanchnics cut, liver nerves not cut, both adrenals in
December 6	24	Light ether, no decerebration, in this and succeeding cases. Splanchnics cut both sides, left upper sympathetic ganglia removed
December 8	14	Splanchnics cut both sides, liver nerves not cut, adrenals in. At autopsy found 2 large strands from right sympathetic cord to semilunar ganglion
December 9	6	Splanchnics cut both sides, liver nerves not cut, adrenals in. Upper abdominal sympathetic chain removed both sides

TABLE 1—*Concluded*

DATE (1924)	MAXIMUM REFLEX ACCELERATION OF HEART	REMARKS
	beats per minute	
December 11.....	24	Splanchnics cut both sides, liver nerves not cut, both adrenals in. After removal of upper abdominal sympathetic chain both sides, reflex increase only 6 beats (168–174); after adrenals tied off, increase, 0
December 18.....	6	Right adrenal removed, left adrenal in, left splanchnics cut, upper left sympathetic chain removed

It should be clearly understood that the operative procedures in these cases left the animals with a satisfactory blood pressure, i.e., above the critical level. The heart rate was recorded on the blood-pressure record.

the heart rate was recorded. Apparently there is no cardiac innervation above the inferior cervical sympathetic ganglion.

The information detailed above naturally led us to conclude that the following operations would assure a heart deprived of all nervous connections with the central nervous system, and that this condition would prevail for possibly two months or more:

1. Removal of both stellate ganglia, thus disconnecting also the inferior cervical ganglia.

2. Excision of several centimeters of the right vagus below the recurrent laryngeal branch and all twigs passing mediad from the vagus, as well as a stretch of the common cardiac nerve.

3. Cutting the recurrent and all other branches of the left vagus between the recurrent and a point below the junction of the vagus with the esophagus.

Known factors affecting the denervated heart. Previous studies have furnished evidence that secretions from the adrenal medulla (Cannon and Rapport, 1921; Cannon and Carrasco-Formiguera, 1922), from the liver (Cannon and Uridil, 1921), and from the thyroid gland (Cannon and Smith, 1922) are each capable of accelerating the beat of the denervated heart—the first two within a few seconds after stimulating the nerve supply, the last after a lapse of minutes and then slowly. The nerves effective in producing these responses belong to the sympathetic division. In removing the stellate ganglia the sympathetic distribution to the thyroid gland would be destroyed. If thereafter the nerves to the liver are cut, and one adrenal gland is removed and the nerves to the other are severed, endocrine agencies influencing the heart rate should be eliminated. Then increase of carbon dioxide in the blood, or asphyxia if sufficiently great,

might be expected to make the heart beat more slowly (Patterson, 1915; Cannon, 1919); and rise or fall of temperature would cause the rate to rise or fall correspondingly (Knowlton and Starling, 1912). If in addition to the influences of the organs mentioned above asphyxia and changes of temperature are ruled out, all the known physiological factors which might influence the activity of the denervated heart would be excluded. The heart then should not be much disturbed, certainly not remarkably accelerated, by the activity of the animal.

To our great surprise the expectation just expressed was not realized in animals surviving for some time after the operations. A few examples will reveal the character of the difficulty which confronted us. The testing of these and other cases consisted in counting the heart rate (by stethoscope), or recording it, while the animal was quiet in the lap, and then placing the animal back downward on a comfortable holder, with the fore legs held by straps and the neck enclosed loosely between two vertical posts united above by a cross bar. Usually sooner or later the animal would begin to pull or jerk about ("struggle") and show signs of rage. This exhibition rarely lasted more than a minute but it was likely to be repeated. If the animal did not become spontaneously active, extending the hind legs moderately, or temporarily closing the nares, would commonly induce the reaction.

Cat 233 had its heart denervated in the manner above described on December 31, 1923. On January 28, 1924, the right adrenal was removed and the left splanchnics cut. When tested February 19, the heart rate increased 28 beats; April 19, 114 beats; May 3, 74 beats—107 to 181 per minute. May 10, the liver nerves were severed. June 16, activity increased the rate from 108 to 184—76 beats per minute. June 24, the left adrenal medulla was sucked out; later the same day activity increased the heart rate 114 beats. July 22, in an acute experiment under light ether, the left vagus was found effective on the heart; but, nevertheless, after both it and the right vagus had been cut, afferent brachial stimulation still accelerated the rate reflexly 46 beats per minute.

The heart of cat 244 was denervated February 4, 1924. February 16 the liver nerves were severed. The right adrenal was removed and the left splanchnics cut April 12. April 24, activity increased the rate 140 beats, from 108 to 248; that afternoon the left splanchnics were again inspected and broken to make sure of their exclusion. April 30, struggle increased the rate from 120 to 244—124 beats per minute. May 3, the vagi were tested in the neck, with no effect on the heart rate. June 16, activity increased the rate 80 beats per minute, 144 to 224. June 19, the left adrenal medulla was sucked out. That afternoon the increase was 84 beats per minute, 160 to 244. In an acute experiment under ether, July 11, both vagi were found effective; but after they were cut in the neck, reflex brachial stimulation increased the rate 28 beats per minute. And when the circulation was confined anterior to the diaphragm, brachial stimulation still accelerated the heart 34 beats, from 204 to 238.

The cardiac acceleration, displayed in the foregoing illustrative cases, after all known nervous and humoral factors had been excluded, proved

to be a very disconcerting and mysterious phenomenon. It was clear that unless it could itself be explained and excluded it might be present whenever any of the known factors mentioned above were acting and thus would vitiate any inferences that might be drawn concerning their effects. We endeavored, therefore, to learn the cause of this belated and unknown accelerating agent.

The search for the unknown accelerator. The appearance of a not previously recognized accelerating agent raised the question of the possibility of finding some indication of its presence in acute experiments. In most of our previous acute experiments on medulliadrenal secretion the animals had been under a general anesthetic; on the other hand, when the unknown accelerator was most effective the animal was not under an anesthetic. It seemed possible that the depth of anesthesia would account for the difference. Accordingly, experiments were planned in which the heart would be denervated under ether, the liver also denervated, the adrenal glands removed or inactivated, the hemispheres destroyed or decorticated quickly through the orbits, and then the anesthetic withdrawn. In all, 14 experiments of this general type were performed. The facts which were newly demonstrated or again emphasized by these experiments were as follows:

1. After removal of the adrenal glands reflex stimulation *may* produce marked acceleration of the heart if the nerves of the liver are intact (October 31, table 1). Therefore an accelerator factor may be given off to the blood from that organ. Section of the hepatic nerves, however, excludes that possibility as a reflex or central nervous effect (Cannon and Uridil, 1921).

2. After the adrenal glands have been removed and the hepatic nerves severed, reflex acceleration of the denervated heart is slight and not readily repeated; in 7 such instances (November 3 to 11, table 1) the maximal reflex increase of rate was 8 beats per minute (1 case, November 6), usually it was much less (4 and 5 beats in 3 cases, November 3, 4 and 5; 2 beats or less in 3 cases, November 7, 10 and 11) (cf. Cannon and Rapport, 1921).

3. If only the splanchnic nerves, major and minor, have been cut, the adrenal medulla is not thereby denervated; strands pass to the semilunar ganglia from upper lumbar sympathetic ganglia on each side (November 13 to December 8, table 1; see also December 11). In 10 such instances the reflex increase of heart rate varied between 8 and 24 beats per minute with only the splanchnics cut: when in addition the upper lumbar sympathetic chain was removed, from the diaphragm to the kidneys, the maximal reflex increase was 6 beats per minute (December 9, 11, 18, table 1) (cf. Stewart and Rogoff, 1917). In other words the conditions were like those seen when the liver nerves are severed and the adrenal glands removed.

4. In the cat there is no crossed influence of the splanchnic impulses of one side on the adrenal medulla of the other side. This was shown not only in acute experiments, but also by testing the right major splanchnic nerve weeks after removal of the right adrenal gland and section of the left splanchnic trunk (see December 2, a.m., table 1) (cf. Elliott, 1912; Stewart and Rogoff, 1916).

The foregoing results confirm earlier observations, made during acute experiments, on the efficacy of nerve section as a means of ruling out hepatic and medulliadrenal activity, and leaving only a small acceleration of the denervated heart. The unknown accelerator did not appear in acute experiments. The secondary large acceleration, which develops in surviving animals after all recognized humoral and nervous agencies affecting the heart rate have been eliminated, is left, therefore, unexplained.

It seemed possible that such long periods between stages of denervation as were common in the early experiments, and as are illustrated in the two cases described on page 334, would permit regrowth of nerve connections at the point first denervated (e.g., the heart) at or near the time when the second denervation was performed (e.g., the adrenals). Obviously this chance for error could be eliminated by performing the complete denervation at once or in two operations near together. This was done in a series of cases, as shown in table 2. The basal rates were those occurring when the animal was quiet in the lap; the increases were due to struggle in the manner already described. It is noteworthy that the increases were small shortly after the operation and became larger as time passed (see especially nos. 172, 16 and 28, table 2). In another animal thus operated upon the rate was 153 beats per minute, when quiet, 24 hours after the operation; there was a maximal increase (recorded by string galvanometer) of only 4 beats per minute when the animal became excited and active. Ten days later, however, the increase was 14 beats per minute; the next day thereafter 18 beats, and the day after that 42 beats. As shown by the results in table 2, after elimination of every known humoral and nervous factor such increases of heart rate as 79, 80 and 84 beats per minute would occur after a struggle. When these results were compared with those which followed corresponding operative procedures in the cases of table 1, the situation became very puzzling indeed. Why should the phenomenon occur in surviving animals and not during an acute experiment?

Among possible explanations for the larger increments of rate of the denervated heart in surviving animals was such a change in the isolated muscle in time as to render it sensitive to influences which do not primarily affect it. A rise of blood pressure does not to a noteworthy degree change the rate of the freshly denervated heart (see Cannon and Rapport, 1921). Can it alter the rate of the denervated heart after a lapse of days? Three days after the denervation the blood pressure was raised, in an acute

TABLE 2

Records of increase of heart rate with activity in animals in which stellate ganglia were removed, right vagus cut below the recurrent branch, left vagal cardiac branches severed, right adrenal gland excised, left splanchnic nerves cut, upper left abdominal sympathetic chain removed, and liver nerves severed under aseptic precautions, in a single operation, or in two operations, as indicated. (Exceptions to the foregoing procedures are mentioned under "Remarks")

CAT NO.	DATE OF DENERVATION		HEART RATES			REMARKS
	Heart	Adrenals and liver	Date	Basal	Increase	
172	Oct. 21, '24	Oct. 15, '24	Oct. 22	144	20	Left adrenal medulla sucked out, Oct. 15
			Oct. 23	156	28	
			Oct. 24	141	51	
			Oct. 25	143	79	Died, Oct. 29, infection
2	Dec. 13, '24	Jan. 15, '25	Jan. 20	150	58	Died, Jan. 22
16	Jan. 23, '25	Jan. 23, '25	Jan. 24	134	6	
			Jan. 26	128	60	
			Jan. 26	180	16	*Acute expt.;* ether, reflex stimulation
				180	20	After cutting right splanchnics
				178	16	After left adrenal tied off
				182	28	Asphyxia of brain
20	Jan. 30, '25	Jan. 30, '25	Feb. 6	204	48	*Acute expt.;* ether, vagi cut in neck
				188	40	After left adrenal tied off
26	Feb. 19, '25	Feb. 19, '25	Feb. 25	120	60	
			Feb. 26	120	60	Increase under atropine
			Mar. 2	100	80	
			Mar. 3	164	52	After left vagus cut in neck
			Mar. 4	148	12	*Acute expt.;* decortication
				164	0	Stimulation right splanchnic
28	Feb. 21, '25	Feb. 21, '25	Feb. 25	128	20	
			Mar. 2	124	52	
			Mar. 10	116	84	Increase under atropine
			Mar. 11	116	44	After thyroids and parathyroids out
				124	52	Increase under atropine
			Mar. 12	120	72	Excited by ether
				132	44	After left adrenal removed

experiment, from 100 to 168 by sudden pressure on the abdomen; the heart rate changed from 178 beats per minute before to 174 during the higher pressure. A similar result was obtained repeatedly in another instance seven days after the denervation. In both cases the animals were decorticated under ether and the tests made after the effect of ether had disappeared. Increased blood pressure did not explain the difficulty.

When an animal struggles muscle metabolites are set free. They have no marked accelerating influence on the rate of the freshly denervated heart (Cannon, Linton and Linton, 1924). They likewise have no accelerating influence when time has passed after the operation. Tests made by stimulating the peripheral end of the cut sciatic in such acute experiments as those just described, 13 and 17 days after the denervation of the heart and adrenals, proved that metabolites do not increase the rate under these circumstances, and therefore that was not the explanation of the mystery.

Again, might not the outlying neurons of the vagus path, still left in place in the heart muscle, assume an autonomous tonic activity when released from control by superior vagus neurons? Consonant with that possibility were paralytic secretion of the submaxillary gland developed after section of the chorda tympani (Bernard, 1864), and the gradually increased tone of the stomach after cutting its vagal supply (Cannon, 1906). Thus the faster rate of the denervated heart might be due to an agent, possibly an internal secretion, inhibiting vagal tone. In our experiments, however, there was no clear indication of the appearance of independent tonic activity of the intracardiac vagal neurons. The rate when the animal is quiet is likely to be somewhat slower as the days pass after the cardiac denervation and the exclusion of hepatic and adrenal influences, but an amount of atropine which fixates the iris neither causes a faster rate nor prevents the acceleration due to struggle (see nos. 26 and 28, table 2). Since atropine blocks vagal impulses, the theory of an inhibited vagus tone had to be surrendered.

The agencies which remained could be classified into endocrine and nervous. The acceleration was too prompt to be due to thyroid influence. Furthermore, it occurred in an animal with thyroids as well as parathyroids removed, in addition to excision of one adrenal (the right) and denervation of the other and also of the liver (see no. 28, table 2). At one time we thought it might be due to an influence from the pituitary, because brief closure of the cerebral vessels was followed by a faster heart rate in an animal deprived of all known accelerator factors. This possibility was made improbable, however, by an experiment in which the cord was transected between the first and second dorsal vertebrae in an animal whose heart, deprived of all known accelerators, had nevertheless been accelerating as much as 76 beats per minute; though the anterior part of

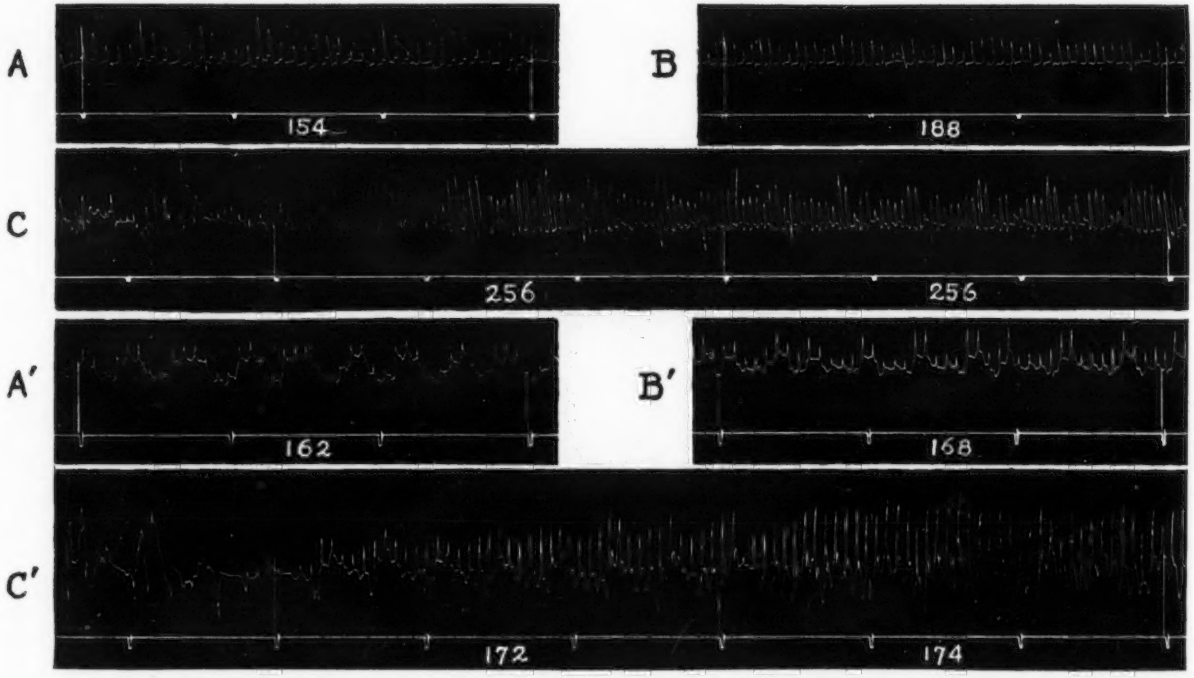

Fig. 2. Records of the heart beat of cat 95 (see table 3). November 2 (a.m.), 1925, after removal of all previously known accelerating agents, the heart rate, when the animal was quiet on the observer's lap, *A*, was 154 beats per minute; quiet on the animal holder, *B*, 188 beats; after struggle (indicated by irregular record) *C*, 256—an increase of 102 beats. January 6, 1926, after removal of upper thoracic sympathetic chains on both sides (November 2, p.m. and December 8), the heart rate, quiet on lap, *A*, was 162; quiet on holder, *B*, 168; and after vigorous struggle *C*, 174—an increase of 12 beats. Time marked in 5-second intervals.

the body, still under control of the brain, was capable of showing excitement and struggle, the heart rate, which dropped from about 120 to 108 beats, could not be made faster by more than 8 beats per minute. It seemed probable, therefore, that the disturbing factor was in the thorax or abdomen. Of the abdominal endocrine organs the gonads were hardly to be suspected of effects on the heart rate. The pancreas had already been shown to be without influence (Cannon and Uridil, 1921). There remained accessory adrenal tissue. This seemed a quite possible cause of the faster beat. The chromophil threads and ribbons associated with the abdominal sympathetic structures contain active adrenin (Vincent, 1910). They are supplied with nerve filaments. It is well known that the iris, deprived of its sympathetic innervation, becomes gradually more sensitive to adrenin secreted into the blood stream; might not the denervated heart likewise become sensitive as time passed? And might not the accessory adrenal tissue gradually hypertrophy because of a deficient supply of adrenin from the main sources? Thus the gradually increasing responsiveness of the heart to excitement and struggle could be reasonably explained. This plausible theory was made untenable when, after inactivation or destruction of the accessory chromophil tissue by removal of both abdominal sympathetic chains in a series of experiments, the heart continued to accelerate just as before. As examples in this series, see records 92, 95 and 110 in table 3.

All possible abdominal causes of acceleration that we could think of have now been reported as having been tested and excluded, except massage of the liver or the remaining adrenal medulla. Actual massage of the liver, more vigorous than would result from contraction of abdominal muscles, failed to reproduce the marked rise of heart rate seen when the animal moved vigorously (cf. November 11 and 13, table 1). And the adrenal factor was excluded, after the liver had been denervated, the right adrenal had been removed and the left adrenal medulla had been sucked out through a cut along one edge of the gland, by observing that the heart could still be made to beat faster (see cases, p. 334 and also no. 172, table 2). The operation was performed with aseptic precautions and allowed repeated tests to be made after the animals had fully recovered their vigor. Thus the evidence pointed to the accelerating agent being below the neck, but not in the abdomen (cf. also cat 244, p. 334).

In the thorax no humoral accelerating factor could be conceived. The cause must be nervous. The stellate ganglia had been removed; they could not mediate the accelerator impulses. Langley (1897) has pointed out a fairly quick renewal of sympathetic connections. Might not preganglionic fibers grow out soon to the inferior cervical ganglia, and might not their axons connect with the heart, and thus explain the faster rate? This somewhat remote possibility was excluded by removing the cervical

TABLE 3

Records of animals in which all hitherto known humoral and nervous accelerators of the heart were removed, and part or all of the abdominal sympathetic chain (cases 92, 95, 97 and 110), without eliminating the faster beat of the denervated heart. Only small increases of heart rate occurred in these cases, and in 4 and 23, when more of the thoracic sympathetic chain was removed. In every instance a section of the common cardiac nerve and of the right vagus trunk below the recurrent laryngeal branch was excised, and the cardiac branches of the left vagus were cut

NUMBER AND DATE	HEART RATE		OPERATIONS AND REMARKS
	Basal	Increase	
(92)			
1925			
September 4....			Right thoracic sympathetic chain removed from ribs 1 to 3; left, 1 to 4
September 18...			Left splanchnic and liver nerves cut, right adrenal and upper 3 left abdominal sympathetic ganglia removed
September 22...	117	36	
October 6.....			Abdominal sympathetic chains removed
October 15.....	112	36	
October 21.....			Right splanchnics cut
October 24.....	112	24	Animal in poor condition
November 25...	156	34	Animal in better health
December 2.....	144	38	
December 8.....			Right thoracic sympathetic chain removed from ribs 3 to 9; left, 4 to 9
December 9.....	100	4	
December 12....	110	6	
December 14....	100	14	
December 18....	130	10	
December 21....	128	12	
December 31....	158	6	
1926			
January 6.....	152	8	
January 19.....	142	24	
January 29.....	154	26	
(95)			
1925			
September 4....			Right thoracic sympathetic chain removed from ribs 1 to 3; left, 1 to 4
September 15...			Left splanchnic and liver nerves cut, right adrenal and upper 3 left abdominal sympathetic ganglia removed
September 16...	152	32	
September 29...	152	72	
October 5.....	148	73	
October 13.....	146	84	
October 14.....			Right splanchnics cut, abdominal sympathetic chains removed
October 19.....	156	84	

TABLE 3—*Continued*

NUMBER AND DATE	HEART RATE		OPERATIONS AND REMARKS
	Basal	Increase	
November 2....	154	102	Remnant of right thoracic sympathetic chain completely removed. (Operation after observation)
November 7....	176	58	
November 18...	172	34	
December 2.....	176	36	
December 8.....			Left thoracic sympathetic chain removed from ribs 4 to 9
December 9.....	152	9	
December 14....	182	12	
December 18....	186	12	
December 21....	172	4	
December 31....	180	8	
1926			
January 6.....	162	12	
January 18.....	144	16	
January 29.....	172	22	
(*110*)			
1925			
September 19...			Right thoracic sympathetic chain removed from ribs 1 to 8, left stellate out
October 14.....			Left splanchnic and liver nerves cut, right adrenal and upper 3 left abdominal sympathetic ganglia removed
October 19.....	137	83	
November 2....	164	72	
November 3....			Right splanchnics cut, abdominal sympathetic chains removed
November 18...	183	49	
December 2.....	172	65	
December 3.....			Left thoracic sympathetic chain removed from ribs 2 to 9
December 4.....	116	8	
December 6.....	152	8	
December 9.....	144	4	
December 14....	124	14	
December 21....	136	8	
December 31....	146	30	
1926			
January 6.....	136	18	
(*97*)			
1925			
July 31.....			Right thoracic sympathetic chain removed from ribs 1 to 4; left, 1 to 8
August 10.....			Left splanchnic and liver nerves cut, right adrenal and upper 4 left abdominal sympathetic ganglia removed
August 12.....	120	8	

TABLE 3—*Concluded*

NUMBER AND DATE	HEART RATE		OPERATIONS AND REMARKS
	Basal	Increase	
August 13.....	99	24	
August 15.....	104	30	
August 20.....	132	24	Lower right thoracic sympathetic chain removed. (Operation after observation)
August 21.....	88	8	
September 1....	92	12	
September 10...	100	10	
(4) 1925			
September 30...			Left splanchnic and liver nerves cut, right adrenal and both abdominal sympathetic chains removed
November 28...			Right and left thoracic sympathetic chains removed from ribs 1 to 8
November 30...	140	4	
December 2.....	152	12	
December 5.....	144	4	
December 9.....	142	8	
December 12....	116	10	
December 18....	120	12	
December 21....	106	10	
December 31....	120	8	
1926			
January 6.....	126	14	
January 29.....	152	32	
(23) 1926			
January 21.....			Right and left thoracic sympathetic chains removed from ribs 1 to 8; liver nerves cut
February 11....			Left splanchnic nerves cut, right adrenal and upper 4 left abdominal sympathetic ganglia removed
February 12....	76	14	
February 15....	78	6	
February 17....	88	8	
February 19....	86	8	
February 23....	104	8	
February 25....	78	10	
March 1.....	114	6	

sympathetic chain on both sides in three animals from which all other known accelerators had already been excluded. Struggle hastened the heart rate quite as much as before.

There remained only the possibility of accessory accelerator fibers from

thoracic sympathetic ganglia below the stellates. As early as November 3, 1924, the promptness of acceleration of the heart, though freed from recognized accelerator influences, suggested that a nervous agent was operating. And on February 6, 1925, when under similar conditions an increase of 40 beats per minute was recorded (see no. 20, table 2) and at autopsy a nerve strand was found passing from a left thoracic ganglion to the arch of the aorta, the possibility seemed confirmed. Accordingly, thereafter the thoracic sympathetic chain was removed down to the third intercostal space on the right side and to the fourth or fifth on the left side. In spite of this addition to the standard operation the acceleration persisted when theoretically it should not. One of these cases manifested an increase of 106 beats per minute when the animal passed from a quiet to an excited and active state, though all splanchnic nerves had been severed, the celiac ganglion excised in addition, the right adrenal gland removed and the left adrenal medulla sucked away! The left thoracic sympathetic chain had been taken out from the stellate ganglion down to the sixth rib inclusive. An extraordinary change occurred in this animal when the right thoracic sympathetic chain was further removed from the third to the ninth intercostal space. The quiet heart rate promptly fell from about 150 beats per minute to about 100, and the increase of rate on exciting the animal fell from 106 to 4. Further decisive evidence was obtained in another case in which the right thoracic chain had been taken out down to the eighth rib and the humoral accelerators eliminated (cat 110; table 3 and figure 3). On the left side the stellate only had been removed. Excitement increased the heart rate as much as 83 beats per minute. Now the left thoracic chain was removed from the second to the ninth rib. The quiet rate dropped about thirty beats and the increase resulting from excitement dropped from the region of 65 to 8 and 14. In still another instance (cat 92, table 3 and fig. 3), in which the quiet rate was about 140 and the increment when the animal struggled was about 40 beats per minute, the right thoracic chain was removed from the third to the ninth rib and the left from the fourth to the ninth; the basal cardiac rate fell to 100 to 110, and the increment fell to 4 to 14 beats per minute. Cat 95, table 3 and figure 3, illustrates further the effect of eliminating the upper thoracic ganglia below the stellates (see also fig. 2). Initial removal of the thoracic ganglia, down to the eighth rib, on both sides in one operation (see cats 4 and 23, table 3 and fig. 3) confirmed these results. With the humoral factors excluded the maximal increase in case 4 during the first four weeks was 12 beats per minute—a minor effect as compared with 70 or 100 beats. Other similar cases have yielded still further support for the conclusion that the mysterious increment of rate was due to accelerator fibers reaching the heart from the sympathetic chain below the stellate ganglia, and even

below the third or fourth thoracic ganglia.[4] More evidence will be reported in later papers.

Perman (1924), in a recent exhaustive article on cardiac innervation, has described fibers passing to the heart in the calf from ganglia III to VI of the left thoracic sympathetic chain, and from ganglia III and IV on the right side, besides the fibers from the stellate ganglia. Similar fibers are known to proceed in man from the upper five or six thoracic ganglia to the cardiac plexus. Although Perman, and Reighard and Jennings (1901) do not record any fibers passing mediad from the sympathetic chain in the cat, it is interesting to note that Mivart (1881) described fine branches given off by the first five or six thoracic ganglia of the cat and distributed "mostly to the aorta and the adjacent parts." The indication that accelerator influences reach the heart from thoracic ganglia below the stellate led us to dissect carefully and with the aid of a dissecting microscope the upper part of the sympathetic chain in more than thirty animals. In nearly all of them fine fibers could be seen passing toward the mid-line from most of the first three or four ganglia below the stellate on either side and occasionally from the fifth and sixth. As a rule it was difficult to trace these fibers to the heart, but in one instance a larger strand than usual was followed to the aortic arch, and in another instance a well-defined strand was found connecting the ganglion below the right stellate with the root of a pulmonary vessel.[5] It seems fairly certain, therefore, that upper

[4] This conclusion with the evidence supporting it was presented at a meeting of the Harvard Medical Society, January 12, 1926 (see Boston Med. and Surg. Journ., 1926, cxciv, 187). The account detailed above gives no indication of the very great difficulties encountered in the course of the investigation. The technical troubles incidental to operating successfully on the chest were themselves serious. Furthermore, after animals had recovered well from operation, they often were afflicted with an epizootic of "snuffles" which made them ill for weeks or caused their death. Thus much valuable time and effort were lost. The minor acceleration immediately following the denervation of the heart, and its gradual development thereafter were most baffling. Naturally the cause could not be discovered by acute experiments. In our bewilderment we had to consider every possible reason for the faster rate and to test every possible agency. The search took, perhaps, much longer than it should. It involved the coöperation of many workers, as indicated by the names at the beginning of this article. Although the "authors" brought the work to a conclusion, the "cooperators," especially J. R. Linton and R. R. Linton, made important contributions to the final outcome.

It is of interest to note that under certain conditions after removal of all the accelerating agencies, struggle caused the heart to beat more slowly. This phenomenon will be considered in a later communication.

[5] Confirmation of these observations was reported in a paper by Dresbach and Waddell (Journ. Pharm. and Exper. Therap., 1926, xxvii, 9) which appeared after our paper was prepared for publication. They found connections between the cardiac plexus and the thoracic sympathetic chain as far down as the fifth ganglion. They

thoracic ganglia other than the stellate send accelerator fibers to the heart, though the fibers are often too small to be readily traced to their destination.[6] In any case the physiological evidence was clear, and the need for removing the upper sympathetic chain in the thorax was evident, if a completely denervated heart was desired. We are now in a position to describe the procedure of denervation.

Procedure of cardiac denervation. Vigorous young male or non-pregnant female cats are selected. After the animal has been deeply etherized a sterilized rubber catheter, 3 mm. in diameter, cut slantingly at the end, is passed through a hole in a wooden stick and introduced into the trachea. The glottis is exposed by separating the jaws with cords slipped between the teeth, and by pulling the tongue forward. A good illumination is necessary for quick action before the deep anesthesia lifts and laryngeal reflexes occur. The stick is placed back of the canine teeth and held there by a thong or strong tape tied back of it and around the jaws. The catheter, reaching to a point a short distance above the bifurcation of the trachea, is fixed in place by a thread fastened tightly around it and now tied to the stick. Thereafter the animal is given artificial respiration by means of an interrupted air blast. The air passes through an ether bottle so arranged that the amount of ether which is added can be nicely regulated. After leaving the ether bottle the air-ether mixture is forced, in cold weather, through a coil of copper tubing which is set in warm water. Thus the air is delivered in the trachea at a temperature approximately normal for natural breathing.

did not present any evidence as to whether these fibers were afferent or efferent In our experience the heart will not be with certainty completely deprived of sympathetic connections, as they state, if the thoracic chain is removed only "as low down as the fourth or fifth ganglion."

[6] Several questions remain to be answered. It is not clear why the heart should accelerate only slightly in acute experiments, and during the first day or two after surgical removal of stellate and vagal influences and exclusion of humoral accelerator factors, and then should gradually respond more and more, as the days pass, to conditions which excite sympathetic activity. It seems possible that removal of the stellate ganglia damages the thoracic chain below the stellates so that it does not function perfectly until it has recovered. Or the heart may be affected by ether and trauma so that it is not so sensitive as it is otherwise. Or possibly the thoracic fibers below the stellate take on a gradually increasing compensatory service after the principal stellate control has been abolished. For the present, however, this question is left unanswered.

Another interesting question is the variation in the basal rate of the denervated heart in different animals and in the same animal at different times (see table 3). There is no indication that the slower rate is associated with persistent vagal effects. This question is set aside for future answer.

A third problem is that of the slight increase in heart rate (4 to 14 beats per minute) after known endocrine factors have been suppressed and thorough cardiac denervation has been performed. Possibly warmer blood brought to the heart would explain the faster rate. We do not now offer, however, any evidence on the point.

The hair is closely clipped from the right and left chest over the fifth intercostal space. These regions are first thoroughly scrubbed with soap and water, next flushed with 95 per cent alcohol, and then with ether; when nearly dry they are wet with a 7 per cent tincture of iodine. Except in these two regions the animal is covered with sterile towels, which in turn are covered with a sterile sheet having an aperture placed over the field of operation. The skin is now incised over the fifth interspace on the right side, and sterile cloths are clipped to the edges. The superficial

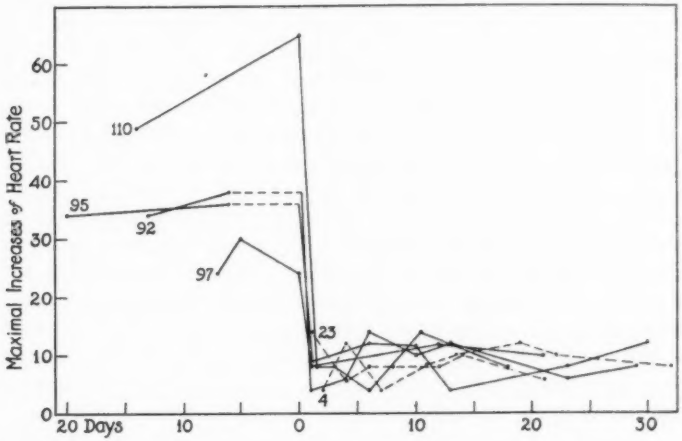

Fig. 3. Graphic representation of the cases reported in table 3. After all hitherto known accelerating agents had been removed the increases of rate of the denervated heart were sharply and markedly reduced in cases 92, 95, 97 and 110 by removal of the remnants of the thoracic sympathetic chain down to the eighth or ninth rib. In cases 4 and 23 (represented by dash-lines) removal of the humoral and nervous accelerating factors, including initial removal of both thoracic sympathetic chains from the first to the eighth ribs, resulted from the outset in only slight increments of the heart rate.

muscles are cut across parallel to the interspace, and the intercostal muscles are broken through with a blunt hemostat while the artificial respiration is temporarily stopped. A sterile silk cloth is introduced to prevent the lung from covering the area of operation at each inspiratory blast, and to protect it from being injured. The fifth and sixth ribs are pushed apart by means of a rib-spreader, and as the spreader widens the gap the intercostal muscles are cut until the opening, which should be extended well toward the back, is about 3 cm. wide. Under protection of a silk cloth the lung anterior to the opening is pressed caudad and is held posterior to the opening and towards the ventral side by means of a flexible retractor inserted between

the arms of the rib-spreader. Thus the fore part and the dorsal aspect of the thoracic cavity are exposed to view.

By means of a blunt dissector, the vagus nerve is uncovered as it courses alongside the trachea. It is grasped in forceps, severed close to the azygos vein, then traced forward to the junction of the accelerator strands from the stellate, and severed again. The part thus isolated is removed. Thus also is excised any branch to the heart that may arise from that portion of the trunk, and the possibility of regrowth of the trunk is minimized. Next the tissue ventral to the trachea is searched until the common cardiac nerve is found. As large an extent as possible of this nerve is excised. All connective tissue ventral to the trachea and between the superior cava, the innominate artery and the trachea is examined carefully for other nerve strands and any indication of a fiber is destroyed. The object of this procedure is to get rid of any adventitious or unusual connections. It is rare to find any. The rib-spreader is now removed and the ribs are held apart and lifted on one side or the other, as necessary, by means of retractors. The sympathetic trunk is exposed on the dorsal wall by opening the pleura with a blunt dissector. The trunk is isolated as far down the chest as possible (at least to the ninth rib); it is then seized in curved forceps and drawn away. It is thereupon traced and isolated forward to the stellate ganglion. The ganglion is grasped in angular forceps, is gently lifted and, with care not to pull upon them, is separated from all its connections by means of long curved scissors. If the branches of the ganglion are not pulled upon, the right recurrent laryngeal branch of the vagus will not be injured and will remain to care for the movements of the right side of the larynx. Transection of the right vagus and the common cardiac nerve and removal of the right thoracic trunk I to VIII have now denervated the heart on the right side. After the silk has been taken out and a slightly increased air pressure has inflated the collapsed lung, the chest is closed, first by a loop of linen thread drawing into their normal positions the fifth and sixth ribs, and then by sewing the different muscle layers together with black silk. The wound is closed by a continuous silk suture, carried in a fine needle, which is pressed through the skin as close as possible to the edge of the cut. A dry dressing of sterile cotton or gauze is temporarily fastened over the wound with strips of adhesive tape.

On the left side a similar opening is made in the fifth intercostal space. The upper lobe of the left lung is gently pressed caudad by means of a silk cloth and it and the other lobe of this region, protected by another silk cloth, are held posterior to the opening and toward the *ventral* side of the chest by means of a curved retractor. The left vagus nerve is uncovered at the point where it joins the esophagus. From that point forward all its branches are cut, up to and including the recurrent laryngeal. The small fiber (depressor) mentioned on page 331 is next severed. The vagus is

then tested electrically in order to prove that all cardiac branches have been disconnected. When that is assured the rib-spreader is removed, the chest wall is lifted by means of retractors, and the left sympathetic chain is removed from the stellate ganglion, inclusive, to the ninth rib, as on the right side. After the silk cloths have been removed and the lung well inflated (it may be necessary to unfold the anterior lobe gently with a blunt dissector), the chest is closed as on the right side. The intratracheal tube should not be withdrawn until the animal is breathing naturally.

The after-care of the animals was directed towards keeping them warm and isolated until they had fully recovered from the ordeal. Immediately after the operation they were placed in a small cage; if the weather was cold they were placed on a covered electric heating pad and also provided with heat reflected from a hot electric coil. By varying the distance of the reflector the temperature of the cage could be quite satisfactorily adjusted to the needs of the animal. After complete recovery from anesthesia water was presented; on the second day milk was offered; and on the third day and thereafter canned salmon, raw liver or cod fish or boiled meat, with milk, were given. When the animals were again in vigorous condition they were allowed to run about a large room.

Methods of recording heart beats. In order to have an objective record of the heart rate a device was necessary which could be employed conveniently and without injury to the animal. At first use was made of the string galvanometer, connected through brine-soaked gauze with a shaven spot on a fore and hind limb. The records were satisfactory, but the method had the defects of being expensive and requiring a trained technician to operate the galvanometer. The method finally adopted was simple, inexpensive and capable of being operated by the observer himself without help.

The apparatus consisted of receiving and recording tambours connected by about 5 feet of small rubber tubing. The receiving tambour was a thistle tube, about 4 cm. in diameter, the mouth of which was covered with a sheet of thin rubber (dental dam or a piece of rubber glove will serve). The recording tambour was provided with a compound lever and was highly sensitive. One or two small pin holes burnt through the rubber of the recording tambour allowed adjustment of the receiving tambour to different surfaces without impairing the sensitiveness of the system to quick, slight pressure changes.

In applying the apparatus the animal is first placed in the situation selected, quiet in the lap or fastened to a holder, and then, after the region of maximal cardiac impulse against the chest wall has been found by palpation, the receiving tambour is held firmly over it. Thereupon with each impulse the writing lever quickly rises and falls. By registering this motion, and the time, on a revolving drum a record of the heart rate is

obtained (see fig. 2). The records vary at different times because the position of the animal shifts and the receiving tambour cannot always be applied in the same place.

The recording apparatus worked best on the flexible chest walls of young cats, for after the ribs have become stiffened with age the cardiac impulse is often not sufficiently prominent to permit good records to be taken. Both vigor to stand the operation and ease of securing cardiac records, therefore, led to the selection of young animals.

Duration of the denervation. Examination of table 3 reveals that in cases 92, 95, 110 and 4, after a period of relatively slight cardiac accelerations, the heart began to respond by larger increments. After 33 days of no greater increase than 12 beats per minute, no. 4 showed an increase of 14 beats on the thirty-ninth day; it rose to 32 on the sixty-second day and to 60 on the eighty-fourth. In nos. 92 and 95 the greater increments began about 40 days after *completing* the denervation. In these cases, as well as in 110, the operation had been performed in several stages (see table 3). A terminal acute experiment on no. 4 proved that the cardiac denervation, done 89 days previously, was still perfect (neither vagal nor thoracic sympathetic stimulation evoked any change); the right splanchnic, however, caused some acceleration, possibly by renewed hepatic connections, though proof was not secured. In a terminal acute experiment on no. 95, stimulation of the right vagus trunk, cleared of remnants of the cervical sympathetic strand, and at a distance of 4 cm. from any other tissue, caused a momentary decrease of heart rate of 32 beats per minute (from 128 to 96) and thereupon, while the stimulation was continuing, an increase of 40 beats per minute (from 96 to 168). After removal of the inferior cervical ganglion the same stimulation decreased the rate 18 beats per minute, and later increased it only 4 beats. The right thoracic sympathetic chain had been removed more than three months previously; the right vagus had been cut below the recurrent nearly six months. It appears probable that the cut vagus fibers had made new connections with both the cardiac vagus neurons and the inferior cervical ganglion. In another instance we found evidence that after four months the inferior cervical ganglia may reconnect with both the heart and with the central nervous system. It is clear that as time passes after denervation very complicated connections may be established between the heart and the central nervous system.

The foregoing cases belonged to a stage previous to the final development of our technic and should be regarded, therefore, as not showing the probable persistence of the denervation. The same comment may be made regarding two cases in which vagal slowing of the heart was demonstrated 68 days after the vagi were deprived of cardiac influence. Nevertheless, because now it appears that regeneration of cut cardiac nerves

may occur within two months or less and thereby may greatly complicate the responses of the heart, the time of observation must be carefully limited. For the present, therefore, it seems prudent to use the heart as an indicator within 30 days after the denervation, and then, by completely eliminating the humoral factors and finding no marked acceleration thereafter, to prove in each case that the heart has in fact been denervated and has remained so.

SUMMARY

The value of the denervated heart in a surviving animal is pointed out. It is a representative organ, existing in the fluid matrix of the body, performing its normal functions, with activity capable of being easily recorded, and influenced only by changes in the blood which perfuses it.

The recognized innervation of the heart of the cat is described (see fig. 1).

When the hitherto recognized nerves reaching to the cat's heart are severed and all known humoral agents (adrenal glands, liver) are excluded from action, the heart nevertheless becomes capable of markedly accelerating if the animal changes from a resting to an excited, active state.

Details are given of the search for the unknown accelerating agent (see tables 1, 2 and 3 and figs. 2 and 3). After elimination of changes in the heart muscle following denervation, and exclusion of one humoral agent after another, as disturbing factors, evidence is presented showing that accessory accelerator fibers from upper thoracic ganglia below the stellates mediate the faster beat. When these are removed, in addition to the removal of the previously recognized agencies causing the heart to beat faster, the heart rate remains steady within approximately 12 beats in spite of vigorous activity (see table 3 and fig. 3).

The details of the procedure for completely denervating the cat's heart are described, as well as the after-care of the animals.

A method for mechanically recording the heart rate of the cat is described.

BIBLIOGRAPHY

BENEDICT, F. G. 1915. A study of prolonged fasting. Carnegie Inst. of Washington, Publ. no. 203, 118.

BENEDICT, F. G., W. R. MILES, P. ROTH AND H. M. SMITH. 1919. Human vitality and efficiency under prolonged restricted diet. Ibid., Publ. no. 280, chart 124.

BERNARD, C. 1864. Journ. de l'Anat. et de la Physiol. Norm. et Pathol., i, 507.

BOEHM, R. AND H. NUSSBAUM. 1875. Arch. f. Exper. Path. u. Pharm., iv, 255.

CANNON, W. B. 1906. This Journal, xvii, 436.

1919. This Journal, l, 399.

CANNON, W. B. AND D. RAPPORT. 1921. This Journal, lviii, 307.

CANNON, W. B. AND J. E. URIDIL. 1921. This Journal, lviii, 354.

CANNON, W. B. AND P. E. SMITH. 1922. This Journal, lx, 476.

CANNON, W. B. AND R. CARRASCO-FORMIGUERA. 1922. This Journal, lxi, 215.

CANNON, W. B., J. R. LINTON AND R. R. LINTON. 1924. This Journal, lxxi, 153.
DOGIEL, J. AND K. ARCHANGELSKY. 1906. Pflüger's Arch., cxiii, 45.
ELLIOTT, T. R. 1912. Journ. Physiol., xliv, 401.
EVANS, C. L. 1912. Journ. Physiol., xlv, 213.
FRANÇOIS-FRANCK, C. A. 1880. Travaux du Laboratoire de Marey, iv, 75.
FRIEDENTHAL, H. 1902. Arch. f. Physiol., 135.
GASKELL, W. H. 1920. The involuntary nervous system, 34.
GUTHRIE, C. C. AND F. H. PIKE. 1907. This Journal, xviii, 28.
HARRIS, J. A. AND F. G. BENEDICT. 1919. A biometric study of basal metabolism in man. Carnegie Inst. of Washington, Publ. no. 279, table 22.
KNOWLTON, F. P. AND E. H. STARLING. 1912. Journ. Physiol., xliv, 206.
LANGLEY, J. N. 1892. Phil. Trans. Roy. Soc. London, Series (B), clxxxiii, 107. 1897. Journ. Physiol., xxii, 215.
MINOT, G. R. AND J. H. MEANS. 1924. Arch. Int. Med., xxxiii, 576.
MIVART, ST. G. 1892. The cat. New York.
PATTERSON, S. W. 1914–1915. Proc. Roy. Soc. London, Series (B), lxxxviii, 380.
PERMAN, E. 1924. Zeitschr. f. Anat. u. Entwicklungsges., lxxi, 382.
REIGHARD, J. AND H. S. JENNINGS. 1901. Anatomy of the cat. New York.
ROGOFF, J. M. 1924. This Journal, lxvii, 551.
SMITH, H. M. 1922. Gaseous exchange and physiological requirements for level and grade walking. Carnegie Inst. of Washington, Publ. no. 309, 170.
STEWART, G. N. AND J. M. ROGOFF. 1916. Journ. Pharm. Exper. Therap., viii, 504. 1917. Journ. Exper. Med., xxvi, 613.
STURGIS, C. C. AND E. H. TOMPKINS. 1920. Arch. Int. Med., xxvi, 467.
TIGERSTEDT, R. 1921. Die Physiologie des Kreislaufes, ii, 388.
VIERORDT, H. 1906. Anatomische, physiologische und physikalische Daten und Tabellen. 3rd. ed., Jena, 44.
VINCENT, S. 1910. Proc. Roy. Soc. London, Series (B), lxxxii, 502.

STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

XVIII. Locus of the Calorigenic Action of Adrenalin with Observations on Tissue Metabolism

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Although it has been known for some time (La Franca, 1909) that adrenalin increases total metabolism, the locus of the rise has not yet been determined. Boothby and Sandiford (1922, 1923) have suggested that the increase is general in distribution but there is little established evidence that a calorigenic stimulation occurs in any tissues other than the heart (Barcroft and Dixon, 1906) and possibly in the central nervous system (Crile and Rowland, 1922), and in skeletal muscle (Ahlgren, 1925). Evidence concerning the action of adrenalin on the metabolism of other tissues is equivocal and largely negative.

Crile and Rowland (1922) and Caskey and Spencer (1925), although with somewhat different results, have used thermoelectric measurements in their efforts to solve this problem. They agree that adrenalin increases the heat production of the brain. Caskey and Spencer found that the heat production of skeletal muscle is also increased by adrenalin, while Crile and Rowland found no change. Crile and Rowland also found that the metabolism of the liver is apparently unresponsive as indicated by the absence of temperature variations. They further report that the increase in brain temperature following adrenalin injection is diminished or absent after hepatectomy.

Martin and Armitstead (1922), using the Haas method of determining metabolism, stated that adrenalin increases the metabolism of isolated skeletal muscle, liver, brain, mesonephros and stomach of the frog as indicated by the rate of total acid production. Griffith (1923) using an improved form of the Osterhout respiration apparatus, concluded that adrenalin as such has no effect on the carbon dioxide production or on the total acid production of the isolated skeletal muscle of the frog. He further pointed out that the slower change in pH which Martin and Armitstead (1922) had observed in their control solutions was really due to the strong buffer action of the bicarbonate which had been added to the con-

trol but not to the adrenalin test solutions. In the face of this criticism the evidence offered by Martin and Armitstead cannot be accepted. Recently Ahlgren (1925) concluded that the oxygen consumption of isolated skeletal muscle of the frog is increased by adrenalin, as indicated by the more rapid reduction of methylene blue solution by that tissue when adrenalin is present.

Masing (1912), working with a perfused rabbit liver, found its oxygen consumption much decreased after the administration of adrenalin in an amount sufficient to give a 1:3,000,000 concentration in the perfusion fluid. This reduction in metabolism probably resulted from a decreased volume flow through the denervated organ, brought about by vasoconstriction (Hartman and McPhedran, 1917). It was shown by McIver and Bright (1924) that the liver may be partially eliminated by tying off its lobes without altering the animal's calorigenic response to adrenalin. This would indicate, at least, that the increase is not limited to the liver.

Some indication as to the distribution of the increase in metabolism can be gained by determining what percentage of the total rise is accounted for by the augmented metabolism of the heart. From the figures of Evans and Ogawa (1914) one may calculate that the increase in oxygen consumption of the Starling heart averaged 65 per cent over a period of twenty minutes directly following the addition of 1 cc. of 1:10,000 adrenalin to the perfusion fluid, which gave a dilution approximating 1:3,000,000. Since total cardiac output is little altered by adrenalin in this concentration (Patterson, 1915; Tigerstedt, 1907), there would occur *in situ* an additional energy demand on the heart due to increase in blood pressure averaging in our own work not over 25 mm. for the twenty-minute period (starting with the injection). According to the calculations of Evans and Matsuoka (1914) this would further increase the oxygen consumption of the heart only 20 to 25 per cent, which makes the average total rise 75 to 90 per cent for the twenty-minute period. The percentage of the total metabolism contributed by the heart has been estimated at 3.6 per cent by Loewy and Von Schrötter (1905) and at 4 to 5 per cent by Evans and Moorhouse (Evans and Matsuoka, 1914). From these figures the average rise in total metabolism resulting from the stimulated oxygen consumption of the heart can be estimated at from 3 to 4 per cent during the twenty-minute period directly following the administration of adrenalin in a 1:3,000,000 concentration.

Since the rise in total metabolism which actually occurs under these conditions of dosage and time has not yet been estimated, we had first to determine that value before estimating the percentage of the whole contributed by the heart. In addition to determining the increase in metabolism of intact animals, we studied the response of animals from which various groups of tissues were partially or completely eliminated.

From these data we hoped to gain some indication of the relative thermogenic stimulation of the various tissues, for if the metabolic response to adrenalin were lowered after the removal of certain tissue it would indicate that the tissue had been specifically active. From these data and the weight of the tissue eliminated we hoped to estimate also the relative rate of metabolism in various tissues as indicated by the ratio of the fall in total metabolism after removal of a part to the weight of the part removed.

METHODS AND TECHNIQUE. The animals used in these experiments were cats in the post-absorptive condition. They were anesthetized with amytal (Page, 1923) given intraperitoneally as a 10 per cent solution in half-normal sodium hydroxide. The initial dose was 50 mgm. per kilo of body weight, but it was necessary to give an additional dose averaging 18 mgm. per kilo in order to maintain surgical anesthesia.

The tissues were eliminated in the following groups by tying their nutrient vessels, the sequence being varied in the different animals:

A. Muscle	Fore limbs	Subclavian arteries at 1st ribs
	Hind limbs	Abdominal aorta, just below renal artery
	Hips	Inferior mesenteric artery
	Lower loins	Inferior epigastric arteries
C. Viscera	Stomach	Esophageal veins
	Spleen	Coeliac trunk
	Pancreas	Superior mesenteric artery
	Intestines	Inferior mesenteric artery
	Colon	Inferior mesenteric vein
	Liver	Portal vein
B. Viscera except liver	As in C,—except coeliac trunk: splenic, left gastric and gastroduodenal branches tied, leaving hepatic artery open.	

Cannon and Cattell, in 1922, concluded from their experimental results that the critical level, that is, the level below which blood pressure is no longer capable of maintaining an adequate volume-flow to the tissues, is approximately 80 mm. of mercury. Below 80 mm. a reduction of the alkali reserve appears, and the animal lapses into shock. Aub in 1920 found that metabolism fell below normal when the blood pressure dropped below 75 to 80 mm. of mercury; in cases of severe shock the fall amounted to about 30 per cent. In order to minimize the complicating effect of shock on metabolism, all ligatures were usually placed before the control period was taken, and about twenty minutes allowed between the completion of the operative procedures and the beginning of the control periods. Experiments on animals with blood pressure below shock level were discarded. As a check on the completeness of elimination the vis-

cera were usually cut loose from their attachments at the time of operation, but left in the abdominal cavity. Occasionally the arteries to the extremities were cut peripheral to their ligatures. Only a negligible amount of bleeding occurred in either case, showing that the elimination was nearly complete. Further evidence that the muscle groups were wholly excluded was the early rigor which developed in the hind limbs and to a slighter degree in the fore limbs.

The adrenalin used was the tablet made by Parke, Davis and Company. As a precaution against deterioration, a fresh solution was prepared for each injection. Two $\frac{1}{200}$ grain tablets were dissolved in 32 cc. (except in the first three experiments) of mammalian Ringer's solution, thereby making a dilution of 1:50,000. In order to adjust the dosage of adrenalin so that it would be comparable to the 1:3,000,000 concentration used by Evans and Ogawa in their Starling heart preparation, it was necessary to allow for the more rapid disappearance of adrenalin when given intravenously. This loss is due, to a small extent, to diffusion through the tissues, but it is more largely due to rapid destruction of adrenalin by the reacting tissues. According to Elliott (1905), and Oliver and Schäfer (1895), adrenalin, in doses such as those given by us, disappears within 1 to .4 minutes after its intravenous administration, and this is further substantiated in our experiments by the return of blood pressure to normal within 1 to 2 minutes after the end of each injection. In the experiments of Evans and Ogawa (1914), considerable adrenalin seems to have persisted twenty minutes after its addition to the perfusion fluid, as indicated by the still elevated rate of the isolated heart (Cannon, 1920). In our experiments two means were used to partially compensate for this more rapid disappearance. The administration was prolonged at an even rate over a period of six minutes, and the total dosage was increased from 0.0167 mgm. to 0.03 mgm. per kilo. As a basis for calculating dilution the blood volume was taken to represent 50 grams of each kilo of body weight (Dreyer, Ray and Walker, 1913). The standard dosage was therefore 0.005 mgm. per kilo per minute which is slightly more than a maximum physiological increase as determined by Cannon and Rapport (1921). In the majority of cases the doses of adrenalin after the elimination of a part were reduced in an amount roughly proportional to the estimated reduction in volume of the circulating blood (Ranke, 1906). The object of this reduction was to keep the doses comparable in the animals before and after the elimination.

The adrenalin solution was injected into a jugular vein from a fine-bore burette connected with a pressure bottle, as shown in figure 1. The rate of flow was roughly controlled by the thumb-screw, *T*, and was more finely adjusted by varying the height of the pressure bottle, *W*. Further details concerning the design and operation of the apparatus are shown in the diagram.

The metabolism was determined by means of a small model of the Roth modification of the Benedict Portable Apparatus (Roth, 1922) designed for small animals. Since the carbon dioxide was removed from the expired air by soda lime, the amount of oxygen consumed was represented by the decrease in volume of gas in the spirometer. The changes in height of the spirometer were recorded by a kymograph during numerous 3- to 4-minute periods which were marked at 5-second intervals by a time-clock signal. From these data, the volume-change having been corrected

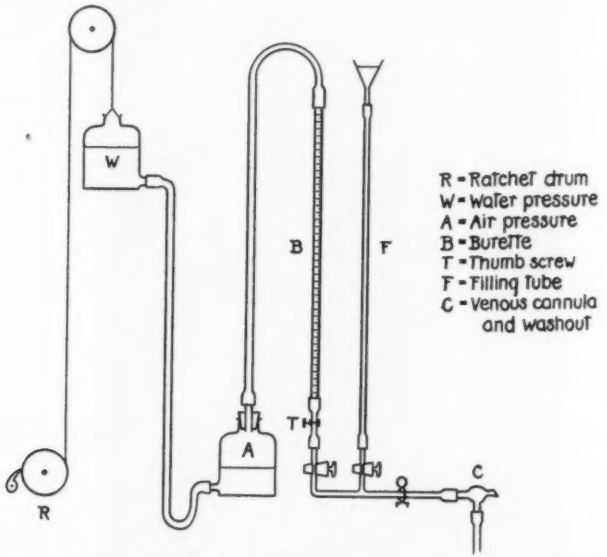

Fig. 1. Diagram of the system used for the injection of adrenalin. The calibrated burette, *B*, was filled through tube *F* by lowering the pressure in *A*. Tube *F* was then closed off and the jugular cannula, *C*, filled with the solution to be injected. The rate of flow into the vein was roughly controlled by the thumb-screw, *T*, and more finely adjusted by varying the height of the pressure-bottle, *W*.

to standard conditions of temperature and pressure, the rate of oxygen consumption was determined and from this figure the rate of metabolism was calculated.

On the kymograph sheet was also traced a simultaneous record of blood pressure. As a further indication of the condition of the animal the rectal or esophageal temperature was entered in the protocol at the end of each period. It was necessary to follow these values closely since slight changes in temperature, or in blood pressure even safely above shock level, alters metabolism to an extent which must be corrected for in this

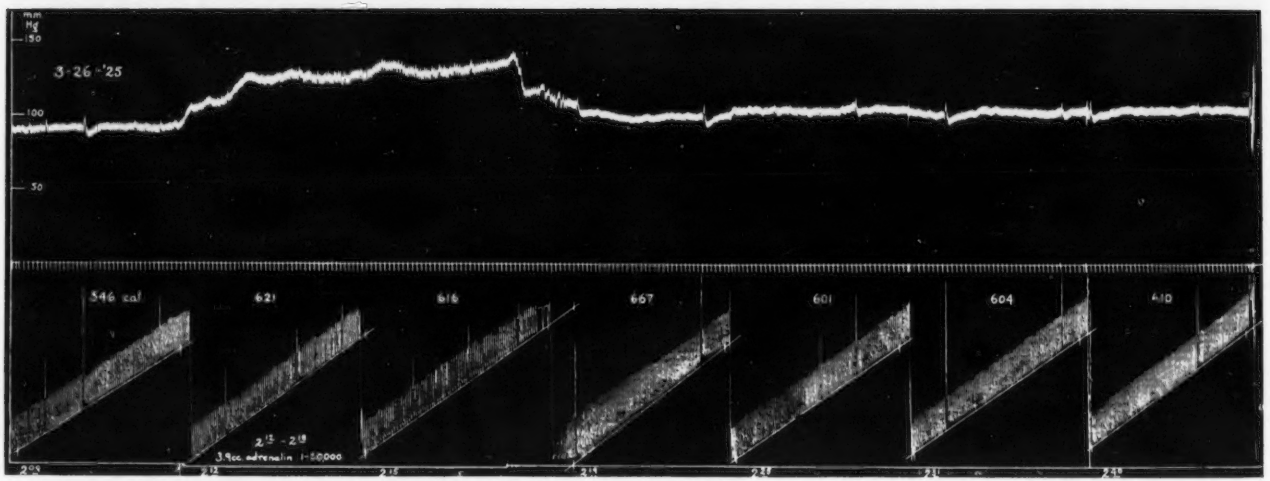

Fig. 2. Kymograph record showing simultaneous records of blood pressure, and of oxygen consumption, from which the metabolism was calculated. The time line, marked in 5-second intervals, is the base line for the blood pressure. The drop in the lowest line shows the duration of adrenalin injection.

TABLE 1

DATE, SEX, WEIGHT, ANESTHETIC, OPERATION, ETC.	ADRENALIN, TOTAL AMOUNT MILLIGRAMS PER KILOGRAMS PER MINUTE	TIME	CALORIES PER HOUR	VARIATION	TEMPERATURE	BLOOD PRESSURE
				per cent	°C.	*mm. Hg*
1/27/25, ♀ 2.55 kgm. Amytal, 50 mgm. per kgm., increased to 68 mgm. per kgm. Vessels to muscles and viscera prepared for ligation. Preparation complete at 3:45		5:27	4.76		37.65	104
		5:30	**4.63**		37.50	106
	5:35–5:41	5:35	5.82	+26.0	37.40	150
	7.05 cc.	5:41	5.54	+20.0		87
	1:100,000	5:50	5.63	+21.8	37.45	98
	[0.0048]	6:05	5.30	+14.6	37.70	185
6:55–6:59 Muscles eliminated by ligating aorta just below kidneys, subclavian, inferior epigastric and inferior mesenteric arteries		7:18	3.80		37.70	100
		7:35	**3.91**	(−15.6)	37.40	118
	7:38–7:44	7:39	4.79	+22.5	37.50	154
	3.85 cc.	7:43	4.81	+23.0	37.50	158
	1:100,000	7:46	4.31	+10.2		130
	[0.0028]	7:53	4.74	+21.0	38.20	138
		7:58	4.71	+20.6	38.30	138
		8:01	4.44	+13.6	38.20	136
8:15–8:28 Viscera including liver eliminated		8:44	**2.33**	(−49.6)	37.00	140
	8:50–8:56	8:53	2.83	+21.4	36.90	180
	3.0 cc.	8:58	2.52	+ 8.0	37.10	124
	1:100,000	9:24	2.03	−13.0	37.10	138
	[0.0021]					
9:30–10:00 Aorta clamped at diaphragm, liver removed		10:06	**1.78**	(−61.5)		100–75
2/3/25, ♀ 3.2 kgm. Amytal 65.0 mgm. per kgm. Vessels to viscera prepared for ligation. Preparation completed at 1:45		2:20	5.05		37.70	120
		2:32	**5.15**		37.60	116
	2:34–2:40	2:35	5.98	+16.0		156
	9.6 cc.	2:38	5.65	+ 9.8	37.45	156
	1:100,000	2:41	4.92	− 4.5	37.45	110
	[0.0050]	2:48	5.46	+ 6.0	37.50	120
		2:55	5.26	+ 2.2	37.55	114
3:12–3:45 Amytal 0.5 mgm. per kgm. Viscera including liver eliminated. (Not included in summary)		4.05	4.01	(−22.0)	37.60	98
		4:15	**3.80**	(−26.0)	37.90	110
	4:20–4:26	4:19	4.27	+12.3	37.95	114
	9.5 cc.	4:23	3.96	+ 4.2	37.98	144
	1:100,000	4:26	3.36	−11.6	38.00	88
	[0.0050]	4:29	2.80	−26.3	38.00	88
2/10/25, ♀ 2.2 kgm. Amytal 75 mgm. per kgm. Vessels to viscera and muscles prepared for ligation. Preparation completed at 1:20		3:19	**4.94**		38.60	126
	3:24–3:30	3:23	4.53	− 8.3		124
	2.6 cc.	3:25	5.59	+13.2		194
	1:40,000	3:27	4.86	+ 1.5		172
	[0.0050]	3:30	5.51	+11.8	38.60	116
		3:34	5.35	+ 8.2		120
		3:40	4.67	− 5.4	38.75	124
		3:45	4.80	− 2.7	38.80	124

TABLE 1—*Continued*

DATE, SEX, WEIGHT, ANESTHETIC, OPERATION, ETC.	ADRENALIN, TOTAL AMOUNT MILLIGRAMS PER KILOGRAMS PER MINUTE	TIME	CALORIES PER HOUR	VARIATION	TEMPERATURE	BLOOD PRESSURE
				per cent	°C.	*mm.* Hg
4:14–4:25 Muscles eliminated		4:44	**4.84**	(− 2.0)	38.45	144
	4:49–4:55	4:49	5.12	+ 6.0		190+
	1.6 cc.	4:52	5.48	+13.2		160
	1:40,000	4:55	5.00	+ 3.4		154
	[0.0031]	5:06	5.11	+ 5.9		160
		5:21	5.14	+ 6.2	38.85	160
5:26–5:32 Viscera, including liver, eliminated		5:43	2.72		37.90	162
		5:48	**2.52**	(−49.0)		162
2/17/25, ♂ 2.7 kgm. Amytal 65 mgm. per kgm. Vessels to limbs and to the viscera prepared for ligation. Thyroids removed. Preparation completed at 12:40		2:22	**6.60**		38.60	80
	2:25–2:31	2:25	8.17	+24.0		148
	4.0 cc.	2:28	7.06	+ 7.0	38.50	160
	1:50,000	2:34	6.64	+ 0.5		96
	[0.0050]	2:37	6.87	+ 4.0	38.20	104
		2:50	6.44	− 2.5	38.30	94
3:19–3:35 Viscera, including liver, eliminated		4:06	**3.88**	(−41.0)		130
	4:10–4:16	4:10	4.94	+27.2		210
	2.35 cc.	4:13	5.05	+30.0	38.50	150
	1:50,000	4.18	4.10	+ 5.8		100
	[0.0031]	4:25	4.47	+15.2	37.95	110
		4:32	4.18	+ 7.8	37.70	106
		4:50	**3.44**	(−48.0)	38.00	100
Weight of parts: Legs and hips, 1270 gm. Liver, 122 gm. All viscera, 365 gm.	4:56–5:02	4:57	4.27	+24.0		170
	2.15 cc.	4:59	4.41	+28.0	37.80	200
	1:50,000	5:02	3.95	+15.0	37.80	78
	[0.0027]					
2/24/25, ♂ 2.1 kgm. Amytal 70 mgm. per kgm. Thyroids removed. Vessels prepared for elimination of muscles and viscera. Preparation completed at 12:30		2:04	**4.55**		37.65	98
	2:16–2:22	2:18	4.71	+ 3.5	38.00	134
	3.1 cc.	2:21	5.11	+12.4	38.00	130
	1:50,000	2:27	5.63	+23.9	37.90	96
	[0.0050]	2:30	4.79	+ 5.2		100
		2:34	4.83	+ 6.0	37.75	100
		3:10	4.57	+ 0.5	37.70	110
3:15–3:24 Viscera including liver eliminated		3:41	**2.83**	(−37.7)	37.80	142
	3:49–3:55	3:50	2.39	−15.5		150
	2.2 cc.	3:54	3.18	+12.3	37.70	146
Weights of parts:	1:50,000	3:58	3.66	+29.2	37.70	128
Legs and hips, 1175 gm.	[0.0035]	4:03	3.50	+23.6	37.60	140
Liver, 60 gm.		4:09	3.35	+18.3	37.50	140
All viscera, 265 gm.		4:17	3.58	+26.6	37.55	138

TABLE 1—*Continued*

DATE, SEX, WEIGHT, ANESTHETIC, OPERATION, ETC.	ADRENALIN, TOTAL AMOUNT MILLIGRAMS PER KILOGRAMS PER MINUTE	TIME	CALORIES PER HOUR	VARIATION	TEMPERATURE	BLOOD PRESSURE
				per cent	*°C.*	*mm. Hg*
2/26/25, ♂ 2.8 kgm. Amytal 86		2:27	**5.63**		36.30	156
mgm. per kgm. Thyroids re-	2:30–2:36	2:30	5.57	− 1.0		170
moved. Vessels to viscera	4.2 cc.	2:34	5.74	+ 2.0	36.40	164
prepared for ligation. Prep-	1:50,000	2:47	5.73	+ 2.0	36.40	165
aration completed at 12:50	[0.0050]	2:52	5.67	+ 1.0	36.40	166
3:20–3:55 Viscera except liver		5:04	**5.49**	(− 2.5)		110
tied off. 4:05–4:25, viscera ex-	5:06–5:12	5:07	5.10	− 7.1	37.70	140
cept liver removed	3.7 cc.	5:10	6.04	+10.0		164
	1:50,000	5:13	6.18	+12.8	37.60	118
	[0.0044]	5:19	5.91	+ 7.8		112
		5:26	5.61	+ 2.2	37.40	114
		5:36	3.84	−30.0	37.20	78
5:40 Liver eliminated: hepatic		5:48	**3.53**	(−37.3)	37.10	78
artery tied	5:54–6:00	5:54	3.11	−12.0		175
	2.7 cc.	6:00	4.56	+29.0		100
	1:50,000	6:04	4.39	+24.2	37.10	76
	[0.0032]	6:09	3.23	− 8.5		66
3/10/25, ♂ 1.8 kgm. Amytal 60		2:53	**4.64**		36.05	82
mgm. per kgm. Thyroids out.	2:57–3:03	2:57	4.81	+ 3.8	36.10	88
Vessels to muscles and viscera	2.7 cc.	3:01	4.65	± 0.0		170
prepared for ligation. Prepa-	1:50,000	3:05	4.74	+ 2.2	36.30	130
ration completed at 12:35	[0.0050]	3:08	4.90	+ 5.8	36.40	78
		3:12	5.00	+ 8.0		97
		3:17	4.90	+ 5.8		98
3:40–3:55 Muscles eliminated		4:05	3.95		36.30	100
	4:14–4:20	4:11	**4.02**	(−13.4)	36.50	100
	2.16 cc.	4:16	4.81	+20.0		170
	1:50,000	4:22	4.27	+ 6.2	36.80	110
	[0.0040]	4:35	4.42	+10.0	36.85	96
3/19/25, ♀ 3.0 kgm. Amytal 65		1:30	**6.48**		38.00	122
mgm. per kgm. Thyroids out.	1:39–1:44	1:40	8.65	+33.8	38.10	190
Vessels to muscles and viscera	3.75 cc.	1:43	9.05	+39.6	38.10	195
prepared for ligation. Prep-	1:50,000	1:52	6.92	+ 7.0		118
aration completed at 12:50	[0.0050]	2:01	7.01	+ 8.3	38.00	114
2:10–3:00 Viscera including liver		3:14	**4.01**	(−38.0)	38.65	146
eliminated	3:23–3:29	3:24	4.39	+9.5	38.40	210
	2.7 cc.	3:28	4.60	+14.6	38.30	210
	1:50,000	3:34	3.98	− 0.6	38.10	110
	[0.0030]	3:53	3.98	− 0.6	38.80	102

TABLE 1—*Continued*

DATE, SEX, WEIGHT, ANESTHETIC, OPERATION, ETC.	ADRENALIN, TOTAL AMOUNT MILLIGRAMS PER KILOGRAMS PER MINUTE	TIME	CALORIES PER HOUR	VARIATION	TEMPERATURE	BLOOD PRESSURE
				per cent	*°C.*	*mm.* Hg
3:58–4:05 Muscles also eliminated		4:25	**3.49**	(−46.0)	38.60	140
	4:30–4:36	4:28	3.53	+ 1.0		190
	2.2 cc.	4:35	4.27	+22.4	38.60	140
	1:50,000	4:45	3.33	− 4.6	38.60	138
	[0.0025]	5:02	3.21	− 8.0	38.90	138
5:06–5:10 Aorta ligated at diaphragm		5:26	**2.35**	(−63.7)	38.35	140
	5:28–5:36	5:30	2.82	+20.0	38.40	200
	2.4 cc.	5:35	2.65	+13.0	38.50	132
	1:50,000	5:40	2,36	+ 0.5	38.60	125
	[0.0020]					
3/24/25, ♀ 2.3 kgm. Amytal 76 mgm. per kgm. Vessels exposed		2:45	4.82		38.10	83
		2:50	**4.90**		38.20	78
	3:02–3:08	3:06	5.24	+ 7.0	38.45	160
	3.45 cc.	3:18	5.76	+18.0	38.50	82
Weight of parts:	1:50,000	3:24	4.98	+ 1.6	38.50	80
legs and hips, 1180 gm., liver, 70 gm., all viscera, 255 gm.	[0.0050]	3:32	4.82	− 1.6		80 shock
3/26/25, ♂ 2.6 kgm. Amytal 87 mgm. per kgm. Thyroids removed. Vessels to viscera prepared for ligation. Preparation completed at 12:05		1:55	5.59		38.45	90
		2:09	**5.46**		38.45	96
	2:12–2:18	2:12	6.27	+15.0	38.45	135
	3.9 cc.	2:19	6.67	+22.0	38.40	102
	1:50,000	2:25	6.01	+10.0	38.40	108
	[0.0050]	2:40	6.10	+11.6	38.40	108
2:43–3:29 Viscera except liver tied off and removed		3:45	5.24		38.80	86
		3:53	**5.52**	(+ 1.0)	38.95*	86
	3:56–4:02	3:57	6.81	+23.5	38.90	142
	3.4 cc.	4:00	6.42	+16.2	38.95	146
	1:50,000	4:05	5.72	+ 3.6	38.95	82
	[0.0044]	4:10	5.99	+ 8.5	38.95	80
		4:25	**5.12**	(− 6.4)	38.90	76
	4:34–4:40	4:35	5.81	+13.6	38.70	138
	3.4 cc.	4:38	5.59	+ 9.0	38.70	138
	1:50,000	4:41	4.95	− 3.4	38.70	60
	[0.0044]					
4/2/25, ♀ 2.65 kgm. Amytal 60 mgm. per kgm. Thyroids out. Vessels to viscera isolated. Preparation completed at 12:10		1:20	6.06		38.35	94
		1:28	**5.96**		38.25	100
	1:36–1:42	1:37	7.27	+22.0	38.40	180
	4.1 cc.	1:40	6.98	+17.0	38.40	160
	1:50,000	1:43	6.67	+12.0	38.15	83
	[0.0050]	1:48	7.05	+18.0	38.10	94
		1:54	6.85	+15.0	38.15	98
		2:09	5.76	− 3.4	38.40	98

*Rise in temperature after operation.

TABLE 1—*Continued*

DATE, SEX, WEIGHT, ANESTHETIC, OPERATION, ETC.	ADRENALIN, TOTAL AMOUNT MILLIGRAMS PER KILOGRAMS PER MINUTE	TIME	CALORIES PER HOUR	VARIATION	TEMPERATURE	BLOOD PRESSURE
				per cent	°C.	*mm.* Hg
2:15–2:42 Viscera except liver eliminated		3:08	**5.33**	(−10.6)	38.60	118
	3:12–3:18	3:14	6.61	+24.0	38.70	200
	4.1 cc.	3:20	5.17	− 3.0	38.60	94
	1:50,000	3:26	5.95	+12.0	38.60	106
	[0.0050]	3:50	5.70	+ 7.0	38.70	104
3:55–4:18 Muscles also eliminated		4:37	**4.49**	(−24.8)	38.20	116
	4:39–4:45	4:41	5.28	+17.8	38.30	220
	3.95 cc.	4:46	5.77	+28.8	38.35	72
	1:50,000	4:51	5.02	+12.0	38.40	98
	[0.0048]	5:01	4.89	+ 9.0	38.50	100
4/9/25, ♂ 2.6 kgm. Amytal 65 mgm. per kgm. Thyroids removed. Vessels to muscles and viscera prepared for ligation. Preparation completed at 12:15		2:32	**6.56**		38.10	106
	2:38–2:44	2:40	7.04	+ 7.2	37.90	158
	3.9 cc.	2:43	7.71	+17.6	37.90	164
	1:50,000	2:46	6.33	− 3.5	38.00	132
	[0.0050]	2:55	6.85	+ 4.2	38.20	114
		3:10	6.78	+ 3.0	38.60	116
3:15–3:20 Muscles eliminated		3:44	**6.30**	(− 4.0)	38.40	170
	3:49–3:55	3:50	7.80	+24.0	38.30	188
	3 9 cc.	3:54	8.46	+34.4	38.30	196
	1:50,000	3:59	6.97	+10.8	38.25	178
	[0.0050]	4:13	6.59	+ 4.6	38.40	186
4/14/25, ♀ 2.0 kgm. Amytal 60 mgm. per kgm. Preparation completed at 12:10		2:01	4.35		37.00	97
		2:21	**4.41**		37.40	92
Later continued as a different experiment	2:23–2:29	2:23	5.30	+20.0	37.40	108
	3.0 cc.	2:25	4.68	+ 6.0	37.55	116
	1:50,000	2:28	4.91	+11.4	37.60	98
	[0.0050]	2:39	4.85	+10.0	37.40	108
		2:56	4.51	+ 2.2	37.10	94
4/22/25, 2.6 kgm. Amytal not good. Injected small amounts till animal was anesthetized. Results not included in averages as cat shivered		3:48	6.35		38.60	98
		3:53	**6.43**		38.60	104
	3:55–4:01	3:57	9.49	+47.5	38.55	195
	3.9 cc.	3:59	8.80	+37.0	38.50	170
	1:50,000	4:01	6.11	− 5.0	38.30	92
	[0.0050]	4:05	6.37	− 1.0		94
5/14/25, ♀ 1.8 kgm. Amytal 60 mgm. per kgm. Thyroids out. Vessels to muscles and viscera isolated. Preparation completed at 12:20		1:43	3.94		36.65	93
		2:05	**3.77**		36.45	83
	2:08–2:14	2:09	4.75	+26.0	36.40	165
	4.1 cc.	2:12	4.79	+27.0	36.50	120
	1:50,000	2:16	3.83	+ 1.5	36.40	88
	[0.0075]	2:19	3.95	+ 4.8	36.30	100

TABLE 1—*Concluded*

DATE, SEX, WEIGHT, ANESTHETIC, OPERATION, ETC.	ADRENALIN, TOTAL AMOUNT MILLIGRAMS PER KILOGRAMS PER MINUTE	TIME	CALORIES PER HOUR	VARIATION	TEMPERATURE	BLOOD PRESSURE
				per cent	*°C.*	*mm. Hg*
		2:25	4.40	+17.0	36.30	110
		2:31	4.47	+18.8	36.30	108
		2:35	4.25	+12.8	36.40	108
2:45–2:58 Muscles eliminated		3:20	**4.05**	(+ 7.4)	36.80	182*
	3:26–3:32	3:26	5.27	+30.0	36.70	178
	3.6 cc.	3:28	4.76	+17.6		170
	1:50,000	3:31	4.37	+ 8.0	36.60	155
	[0.0067]	3:35	4.33	+ 7.0	36.45	150
		3:57	4.46	+10.0	36.85	144
		4:03	**3.95**	(+ 4.6)		150*
	4:06–4:12	4:06	4.97	+26.0		169
	3.6 cc.	4:08	5.17	+31.0	37.20	174
	1:50,000	4:10	4.84	+22.4	37.20	166
	[0.0067]	4:15	4.41	+11.8	37.20	148
		4:20	4.37	+10.8	37.30	175
		4:33	4.16	+ 5.4	37.15	150
		4:51	**3.99**	(+6.0)	37.20	136
	4:52–4:58	4:55	4.37	+ 9.6	37.30	154
	3.6 cc.	5:02	3.70	− 7.3		140
	1:50,000	5:13	3.83	− 4.0	37.50	140
	[0.0067]					
5/19/25, ♀ 1.8 kgm. Amytal 60 mgm. per kgm. Thyroids removed. Vessels to muscles and viscera prepared for ligation. Operation completed at 12:00		2:32	**5.13**		37.20	84
	2:35–2:41	2:36	5.72	+11.5	37.30	150
	2.7 cc.	2:39	5.77	+12.6	37.30	140
	1:50,000	2:44	4.87	− 5.0	37.15	84
	[0.0050]					
3:12–3:18 Muscles eliminated		3:32	**4.97**	(− 3.0)	36.95	146*
	3:35–3:41	3:35	5.61	+13.0	37.10	172
	2.5 cc.	3:38	5.43	+ 9.0	37.20	168
	1:50,000	3:42	5.17	+ 4.0	37.30	148
	[0.0046]	3:48	5.43	+ 9.0	37.40	168
		3:55	5.47	+10.0	37.55	176
		4:05	5.22	+ 5.0		178
		4:16	**4.97**	(− 3.0)	37.60	180
	4:18–4:24	4:21	5.63	+13.0	37.60	185
	2.5 cc.	4:31	5.14	+3.4	37.60	174
	1:50,000	4:39	5.41	+ 9.0	37.60	174
	[0.0046]	4:52	4.99	− 0.5	37.70	168
		4:56	4.81	− 3.2	37.70	176

*Note increased blood pressure.

work. Du Bois (1921) found that in fever there is a 13 per cent increase in metabolism for every degree rise in temperature above the normal, and Aub and White (unpublished) have found that a rise in temperature of 1.0°C. increases a cat's heat production by about 10 per cent. In our work the temperature of the animals was well controlled by means of an electric heating pad, the temperature rarely varying more than half a degree centigrade, and never, even over a period of several hours, more than a degree. From the data of Evans and Matsuoka (1914) one may calculate that a rise in blood pressure of 20 to 25 mm. raises the total heat production by one per cent, by increasing the work of the heart. All

SUMMARY TABLE 2

Metabolic rate in parts eliminated

ELIMINATED PARTS	NUMBER OF CASES	BLOOD PRESSURE		TEMPERATURE		REDUCTION IN METABOLISM		REDUCTION IN WEIGHT	METABOLIC RATE (CALCULATED)
		Basal	Change after elimination	Basal	Change after elimination	Measured	Corrected		
		mm. Hg	*mm. Hg*	*°C.*	*°C.*	*per cent*	*per cent*	*per cent*	*cal./kgm./hr.*
Normal.....	15	102		37.61					2.18 (entire cat)
A. Legs and hips (muscles)	8	92	+40	37.33	+0.05	−6.8	−9.3	−50.0	0.40 (legs and hips)
								−30.0	0.60 (muscle)
B. Viscera, except the liver..	3	112	−16	37.36	+0.70	−5.3	−11.5	−9.0	2.90 (viscera except liver)
C. Viscera, including liver..	8	109	+27	37.64	+0.30	−38.3	−41.5	−12.5	7.35 (all viscera)
							−30.0	−3.5	18.70 (liver)

corrections in metabolism made to compensate for changes in temperature or blood pressure (see table 2) have been carried out according to these figures.

DATA. The tables of the individual experiments are arranged in chronological order. In the first column of each table are entered brief descriptions of the cats, the amount of amytal given and the operations performed. In the second are stated the amounts of adrenalin, the figures in brackets interpreting these values in terms of ten-thousandths of a milligram of adrenalin per kilo of body weight per minute. In the third column is a record of the times at which the three-minute metabolism periods were started. In the fourth column is expressed the total metabo-

lism in calories per kilo per hour, the basal periods in bold face type. In the next column is the calculated percentage variation from the preceding basal. Percentages placed in parentheses, on a line with the basal periods, represent a change from the original basal due to the elimination of certain tissues. The temperature and blood pressure are recorded in the last two columns. Only a few results of giving adrenalin after eliminating both the limbs and the viscera including the liver have been tabulated here, since these animals usually failed quite rapidly, as shown in the experiment of January 27, 1925.

The summary tables present the average conditions of blood pressure, temperature and metabolism which occur under similar conditions of tissue elimination and dosage of adrenalin. Table 2 shows the changes follow-

SUMMARY TABLE 3

Response of entire and of reduced animals to adrenalin

ELIMINATED PARTS	NUMBER OF CASES	ADRENALIN 6-MIN. DOSE MGM./KGM./MIN.	BLOOD PRESSURE		AVERAGE RISE IN METABOLISM	
			Basal	Increase	Peak	20-minute
			mm. Hg	*mm.* Hg	*per cent*	*per cent*
Normal	14	0.0050	102	57	19.0	10.6
	1	0.0075	83	85	27.0	18.0
A. Legs and hips (muscles)	6	0.0042*	144	34	19.4	12.4
	3	0.0067	157	14	23.5	12.5
B. Viscera, except the liver	3	0.0044	91	58	16.7	9.0
	1	0.0050	112	82	24.0	11.0
C. Viscera including liver	6	0.0031*	140	70	23.9	12.0

* Average.

ing the elimination of various groups of tissues. In the next to the last column the percentages of total body weight represented by the tissues eliminated are based on measurements made in the experiments of February 17, February 24 and March 24, 1925. Table 3 deals with the effects of adrenalin on the blood pressure and metabolism of entire and reduced animals. The figures in the last two columns give the percentage increases in metabolism resulting from adrenalin,—the first of these showing the average peaks of the rises, and the last, the average general rises during the twenty minutes directly following the beginning of the adrenalin injection.

Discussion. *A. Effect of amytal on metabolism.* Aub, Bright and Uridil (1922) found that the standard metabolism of normal cats is 2.28 calories per kilo per hour at an average temperature of 39.2°C. They

also showed that urethane anesthesia increases metabolism about 13 per cent by stimulating the sympathetic nervous system and the adrenal glands. We have found that the metabolism of cats under amytal anesthesia averages 2.18 calories per kilo per hour at an average temperature of 37.6°C. Correcting for the discrepancy in temperature of 1.6°C., according to the findings of Aub and White (unpublished), the basal rate would become 2.53 calories per kilo per hour. This is equivalent to an increase in metabolism of over 10 per cent. In view of the general lack of stimulation of the sympathetic system by amytal which Page reported in 1923, and which Britton confirmed in 1925, this increase was not expected.

B. Relative rate of metabolism in various tissues. Although our work gives no indication of the metabolic rate of heart muscle alone we shall, by way of introduction, refer to the work of Evans (1912). He estimated that the resting heart consumes 3.5 to 6.0 cc. of oxygen per gram of weight per hour. According to the table of Carpenter (1921), this is equivalent to a metabolic rate of 17 to 30 calories per hour.

The metabolic rate of skeletal muscle has been variously estimated. Smith, in 1884, measuring the difference in temperature of blood in the arteries and veins of muscles, determined the heat production of that tissue to be 0.41 calorie per kilo per hour. Von Frey and Gruber (1885) estimated the metabolism of perfused dog muscle at 0.58 calorie per kilo per hour. Verzár (1912) determined the oxygen consumption of the gastrocnemius of the cat to be 0.00448 cc. per gram per minute which is equivalent to a heat production of 1.3 calories per kilo per hour. Our results would indicate that the rate of metabolism in the legs and hips (including bone, skin and muscle) is about 0.40 calorie per kilo per hour. Assuming that the oxygen consumption of such inactive tissues as bone and skin would be very low (0.10 calorie per kilo per hour), and knowing that muscle represents approximately 50 per cent of the total mass of the extremities (Vierordt, 1906), we can roughly estimate the metabolism of muscle alone to be about 0.60 calorie per kilo per hour.

Cohnheim and Pletnew (1910) showed that perfused small intestine produced 0.40 to 0.45 cc. of carbon dioxide per hour per gram of tissue, which is equivalent to a heat production of 2.50 calories per kilo per hour (Carpenter, 1921). Our results indicate that the heat production of a composite, consisting of stomach, intestines, pancreas and spleen, is 2.9 calories per kilo per hour, corrections having been made for changes in temperature and blood pressure. This value is probably high, since the operation would necessarily cut off the portal flow into the liver and thereby interfere with its metabolism to some extent.

Masing (1912) showed that a perfused rabbit liver used 1.5 to 2.4 cc. of oxygen per minute per gram of tissue. This is equivalent to a heat pro-

duction of from 4.35 to 7.0 calories per kilo of tissue per hour. Our experiments gave an average fall in metabolism of 41.5 per cent after the elimination of the liver and other viscera, while after the elimination of all except the liver it fell only 11.5 per cent. This leaves a decrease of 30 per cent attributable to the eliminated liver, which indicates that the liver is a very important factor in the height of total metabolism. In case that fall represents only that portion of total metabolism contributed by the liver itself, its heat production would average about 18.7 calories per kilo per hour. It seems more plausible to ascribe part of this fall to a depression in general metabolism due indirectly to trauma.

C. Calorigenic action of adrenalin. In entire animals the peaks of the rises in metabolism resulting from the injection of 0.0050 mgm. of adrenalin per kilo per minute for six minutes averaged 19.0 per cent. This confirms the finding of Boothby and Sandiford (1922) and of McIver and Bright (1924). The period of highest metabolism in many cases parallels or closely follows the rise in blood pressure, but can in only small measure be due to that rise. As previously pointed out, an increase in blood pressure of 20 to 25 mm. in the range above the critical level (Cannon and Cattell, 1922), raises the total metabolism by only 1 per cent. The peaks of the rises in blood pressure of intact animals averaged 57 mm. which would raise total metabolism not more than 2 or 3 per cent. The relative independence of these phenomena is further indicated by the results of individual experiments, which are cited as exceptions rather than as the rule. The records for March 10, 1925, show marked blood-pressure reactions with slight alterations of metabolism, while those for May 14, 1925 show rises in metabolism occurring both with and without rises in blood pressure. The metabolism of the artery walls should not be increased during contraction since the contraction of other smooth muscle is attended by little or no increase in its heat production (Snyder, 1914). During the entire twenty-minute period directly following adrenalin the general rise in metabolism averaged 10.6 per cent. Of this total rise of 10.6 per cent only 3 to 4 per cent, that is, about one-third, is accounted for by the increased metabolism of the heart (Evans and Matsuoka; Evans and Ogawa, 1914). This is conclusive evidence that adrenalin has a calorigenic action on tissues other than the heart and circulatory system.

The relative response to adrenalin was little altered after large muscle masses had been eliminated by ligating the arteries to the hips and extremities. In the animals from which the viscera except the liver had been eliminated, there was a lack of close correspondence between the reactions but we believe that the average response to adrenalin is practically unaltered. After eliminating the viscera including the liver, both the average general rise in metabolism and the peaks of the rises are increased, although the dose of adrenalin had been reduced 38 per cent. The peak

was increased proportionately more than the general level, suggesting that the change was due to the magnification of an intense but brief response such as the heart would give. Accordingly we interpret this alteration as due to a relative accentuation of the local increase in the heart as a result of the 40 per cent reduction in total metabolism which followed elimination of the visceral organs.

The approximate uniformity of the percentage rises in metabolism of entire and reduced animals indicates that the response is not concentrated in any of the eliminated tissues,—neither in the extremities nor in the visceral organs,—but is general in distribution, as suggested by Boothby and Sandiford (1922, 1923). It is true that this calorigenic response to adrenalin is to some degree emphasized in the heart, and it does not appear to result entirely from the increased work (Evans, 1917).

It gives us great pleasure to acknowledge our gratitude to Dr. Walter B. Cannon for his inspiration and guidance in this work.

CONCLUSIONS

Amytal anesthesia increases the resting metabolism of the cat about 10 per cent.

The basal rate of metabolism in muscle is low, 0.50 to 1.0 calorie per kilo per hour. In the liver it is high, 10 to 20 calories per kilo per hour. In the other viscera it averages 2 to 3 calories per kilo per hour.

Adrenalin given in a dosage of 0.005 mgm. per kilo per minute for six minutes, produces rises in total metabolism which average 10.6 per cent during the following twenty-minute period. The peaks of the rises average 19.0 per cent.

The increased metabolism of the heart would account for only one-third of this total rise.

Adrenalin has a general calorigenic action on tissue metabolism.

BIBLIOGRAPHY

AHLGREN, G. 1925. Skand. Arch. f. Physiol., xlvii (supplement).
AUB, J. C. 1920. This Journal, liv, 388.
AUB, J. C., E. M. BRIGHT AND J. FORMAN. 1922. This Journal, lxi, 349.
AUB, J. C., E. M. BRIGHT, AND J. URIDIL. 1922. Ibid., 300.
AUB, J. C., J. FORMAN AND E. M. BRIGHT. 1922. Ibid., 326.
BARCROFT, J. AND W. E. DIXON. 1906. Journ. Physiol., xxxv, 182.
BOOTHBY, W. M. AND I. SANDIFORD. 1922. This Journal, lix, 463.
1923. Ibid., lxvi, 93.
BRITTON, S. W. 1925. This Journal, lxxiv, 291.
CANNON, W. B. 1920. This Journal, l, 399.
CANNON, W. B. AND M. CATTELL. 1922. Arch. Surgery, iv, 300.
CANNON, W. B. AND D. RAPPORT. 1921. This Journal, lviii, 308.
CARPENTER, T. M. 1921. Carnegie Inst. of Washington, Publ. 303, p. 104.
CASKEY, M. W. AND W. P. SPENCER. 1925. This Journal, lxxi, 507.

COHNHEIM, O. AND PLETNEW. 1910. Zeitschr. f. Physiol. Chem., lxix, 96.
CRILE, G. W. AND A. F. ROWLAND. 1922. This Journal, lxii, 370.
DREYER, G., W. RAY AND E. WALKER. 1913. Skand. Arch. Physiol., xxviii, 299.
DU BOIS, E. F. 1921. Journ. Amer. Med. Assoc., lxxvii, 352.
ELLIOTT, T. R. 1905. Journ. Physiol., xxxii, 441.
EVANS, C. L. 1912. Journ. Physiol., xlvii, 446.
1917. Ibid., li, 91.
EVANS, C. L. AND Y. MATSUOKA. 1914. Ibid., xlix, 378.
EVANS, C. L. AND O. OGAWA. 1914. Ibid., xlvii, 446.
GRIFFITH, F. R., JR. 1923. This Journal, lxv, 15.
HARTMAN, F. A. AND L. MCPHEDRAN. 1917. This Journal, xliii, 311.
LA FRANCA, S. 1909. Zeitschr. f. Exper. Path. u. Therap., vi, 1.
LOEWY, A. AND H. VON SCHRÖTTER. 1905. Zeitschr. f. Exper. Path. u. Therap., i, 197.
MARTIN, E. G. AND R. B. ARMITSTEAD. 1922. This Journal, lxii, 488.
MASING, E. 1912. Arch. f. Exper. Path. u. Pharm., lxix, 431.
MCIVER, M. A. AND E. M. BRIGHT. 1924. This Journal, lxviii, 622.
OLIVER, G. AND E. A. SCHÄFER. 1895. Journ. Physiol., xviii, 273.
PAGE, I. H. 1923. Journ. Lab. and Clin. Med., ix, 3.
PATTERSON, S. W. 1915. Proc. of the Royal Soc. of London, B-88, 371.
RANKE, J. 1871. Die Blutvertheilung und der Thätigkeitswechsel der Organe, s. 80.
1906. Vierordt's Daten u. Tabel. f. Mediz., s. 194.
ROTH, P. 1922. Boston Med. and Surg. Journ., clxxxvi, 457.
SMITH, R. M. 1884. Arch. f. (Anat. u.) Physiol., 261.
SNYDER, C. D. 1914. This Journal. xxxv, 340.
TIGERSTEDT, R. 1907. Skand. Arch. f. Physiol., xix, 1.
VERZÁR, F. 1912. Journ. Physiol., xliv, 243.
VIERORDT, H. 1906. Daten u. Tabel. f. Mediz., s. 44, 184.
VON FREYM, AND M. GRUBER. 1885. Arch. f. (Anat. u.) Physiol., 519.

STUDIES ON THE MOTILITY OF THE DENERVATED MAMMALIAN ESOPHAGUS

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LITERATURE. Without doubt, the chief extrinsic nerves controlling the esophagus of vertebrates are the vagi. According to some, the sympathetics also share in the control of this region of the gut.

The investigations carried out are chiefly concerned with an attempt to find or analyze the kind of fibers, whether motor or inhibitory, that are contained in the trunks of the nerves supplying the esophagus of both lower vertebrates as well as those of mammals.

Investigations along these lines have been made upon amphibia (chiefly frogs) by Ravitsch (31), Goltz (14), Waters (47), Steinach (42), Bottazzi (3) (4), Hopf (16), Patterson (29), Carlson and Luckhardt (10); on reptiles (turtles) by Bercovitz and Rogers (1), Carlson and Luckhardt (10); and on birds (pigeon) by Doyon (13).

Cannon (6) reports that Reid (32) noticed in 1839 stasis of food in the esophagus after vagus section. Reid (32) found stasis in the rabbit; but, he reported, that "substances seem to pass pretty freely along the oesophagus in most dogs after section of vagi."

Bernard (2) in 1849, and Schiff (37) in 1867 noted that vagotomy in dogs leads to temporary spasm of esophagus and cardia. A paralysis of esophagus of horses following double vagotomy is reported by Chauveau (11) in 1862.

Openchowski reported (27, 28) in 1889 a special dilator nerve for the cardia of rabbit, dog and cat. He also noticed Auerbach's plexus and special ganglion cells in the cardia, and that constrictor fibers of the cardia occur in the vagi (mainly in the left vagus), as also in the cervical nerves and cervical sympathetics. Dilators reach the cardia by way of the thoracic sympathetic trunks passing to the cardia through the aortic plexus, splanchnics and gastric plexus.

Langley (20) claims that the vagi of rabbit contain both motor and inhibitory fibers to cardia. The sympathetics also give motor and inhibitory effects on the esophagus (22).

Krehl (18) in 1892 found that vagotomy in a dog causes the esophagus and cardia to be atonic. A stimulation of the splanchnics in dogs gave Morat (26) motor effects on stomach.

Sinnhuber (38) in 1903 claimed that vagotomy in the dog, if performed above and close to the diaphragm, causes the cardia to be atonic; whereas vagotomy in the neck induces only a temporary hypertonicity, which is interpreted by Carlson (10), "in the dog the inhibitory fibers to cardia leave the vagi at some distance above the diaphragm and pass down to the cardia in the wall of the esophagus."

Starck (41) in 1904 reported that vagotomy in dogs relaxes the esophagus and slightly increases the tonicity of the cardia. Page May (23) concluded that splanchnics had no action on cardia or stomach, and asserted that all so-called splanchnic effects were due to escape of current or stimulation of the diaphragm. Inhibiting fibers to the cardia in the vagi of rabbits were demonstrated by Meltzer and Auer (25) in 1906. Spandolini (40) noted in 1916 that the splanchnics yield both motor and inhibitory effects on the stomach of dog. Viewed from the point of adrenalin action Smith (39) in 1918 reported motor and inhibitory effects on strips of stomach, especially in man and cat, as also in certain other mammals.

Schafer (35) doubly vagotomized cats and observed vomiting. He reports intussusception of the abdominal viscera into the esophagus of cats that died presumably of asphyxia.

Stimulation of the vagi in a monkey was found to produce contraction in the cardia by Carlson and Litt (9). Both motor and inhibitory vagi efferents to the cardia and esophagus in cat were found by Carlson (7) in 1922. The end result of stimulation, whether relaxation or constriction, of cardia was the reverse of the tonic condition of the sphincter at the time. He reports that "splanchnic nerves carry both motor and inhibitory efferents to the cardia and esophagus in the cat. In dogs only motor action, in the rabbit only inhibitory action was demonstrated. These variations are probably due to the condition of the cardia. . . . . "

Veach (45) reported that "Stimulation of the peripheral end of either vagus nerve with relatively low frequencies or intensities has motor effects on the lower end of the esophagus, the cardia, and the body of the stomach of the cat. Stimulation with considerably higher frequencies or intensities has inhibitory effects on these structures. . . . " These results he interprets as being of the same nature as the Wedensky inhibition. Howell (17) criticizes this view. Stimulation of splanchnics in cats, Veach (46) says, results in motor effect on the lower end of esophagus, "though frequently" this effect "is apparently lacking."

Cannon (6) states that: "In 1846 Wild reported experiments which showed that if the esophagus is divided or merely has a thread tied tightly about it, the peristaltic wave is definitely blocked at the point of interference. From this observation he drew the conclusion that esophageal peristalsis is due to a series of reflexes starting in the mucous membrane of the esophagus itself—a series at once stopped by any interruption of the

continuity of the tube." Mosso "placed a small wooden ball in the esophagus below the point where the tube had been transected. When a wave started by a swallowing movement had traversed the upper section it did not stop at the point of incision but in due time appeared below and carried the ball to the stomach." Mosso concluded "esophageal peristalsis is originated step by step in the central nervous system." The experiments of Wild and Mosso were repeated by Meltzer (24). "According to Meltzer's results Wild observed conditions which appear in deep anesthesia and discovered the reflex peristalsis which can originate in the esophagus itself. Mosso on the other hand who studied the condition in light anesthesia discovered the central origin of the procession of esophageal peristalsis which normally prevails." "Mosso's observation revealed an esophageal peristalsis of central origin, distinguished by Meltzer as primary peristalsis. Wild's studies disclosed a reflex esophageal peristalsis of peripheral origin, the secondary peristalsis of Meltzer."

Cannon (5) prepared cats for double vagotomy by the Pavlov method. He observed paralysis of the whole esophagus immediately after operation, recovery of the lower end, and noted that this corresponds to the smooth musculature. The peristaltic activity in the lower smooth end of the esophagus, capable without vagus support to clear the esophagus of food, may be denoted as the tertiary peristalsis of Cannon. These experiments repeated on the monkey gave identical results as in cat; but when tried on the rabbit, no tertiary peristalsis were in evidence, since the esophagus of rabbit, unlike that of cat and monkey, is composed wholly of striated muscle.

Noting the effect of double vagotomy upon esophagi composed in part of smooth musculature, it was thought desirable to extend these studies. In the first place, it was queried what will be the behavior of the striated musculature in an esophagus of a doubly vagotomized animal in case the vagi do not regenerate. Further, in case the striated musculature does not atrophy, can it regain some automatic activity? Again, since the splanchnics have been shown by Carlson, also to supply the lower end of the esophagus, what effect would their lesion have upon this organ? Moreover, it was thought that, perhaps, the results of this study might cast some light upon cardiospasm in man. Hence the investigation was entered upon at the instigation and direction of Professor Carlson.

METHODS OF STUDY. Cats that have been prepared for this study of the motility of the denervated mammalian esophagus, were observed with the aid of a fluoroscopic screen over x-rays while feeding upon a paste consisting of minced liver, barium sulphate, and water.

Double vagotomy. This operation, as is well known, requires a special precaution against asphyxia "owing to the falling together in inspiration of the thyro-arytenoid ligaments and arytenoid cartilages, the time of onset and duration of the asphyxiation varying in different species of animals and in

different individuals of the same species" (35, 36). Occasionally, few individuals survive indefinitely. This asphyxia can be obviated by the employment of either one of two methods. Pavlov cuts the left vagus in the neck and the right vagus below the point (in the thorax) where the inferior (or recurrent) laryngeal has been given off. Schafer (35) performs an electrocautery of the larynx and cuts the vagal trunks on both sides in the neck.

Inasmuch as the inferior laryngeal nerve also innervates the esophagus (15, 19), it was desirable to include this branch of the vagus in the section; hence Schafer's method was resorted to. In order to avoid unusual operative risks, only cats in good nutrition were selected. The choice of an anesthetic for the cautery is not arbitrary. For when ether is used in electrocautery of the larynx, unless special care is exercised, there is danger of the ether vapors igniting, and thereby causing injury to the trachea and bronchi as a result of the backflash. I have found that barbital-sodium (0.25 gram per kilo of body weight) with cocainization (2 per cent cocain and few drops of 1/1000 adrenalin) of the larynx, although easily administered to dogs by means of a stomach tube, presents special difficulties in the case of cats, requiring a number of assistants to pass the tube, owing to their resistant efforts. However, cats readily lap up the required dose of barbital-sodium in milk. But the period of anesthesia is too long. Hence in spite of its possible toxic effects, chloroform, with a subcutaneous injection of atropine sulphate (0.3 mgm.) as a precaution against salivation and mucus secretion, gave me the most satisfactory results of all the anesthetics that I have tried.

As soon as deep anesthesia has set in, the mouth of the animal was opened wide, the tongue and epiglottis pulled forward and down, and held so by a tongue depressor, whereupon the electrocautery was introduced through the mouth. The ligaments were then burned with the cautery needle red hot.

Allowing a few weeks for recovery, the cat was then prepared for double vagotomy, using ether or chloroform as an anesthetic, with or without subcutaneous administration of atropine sulphate. Then with general aseptic precautions both vagi and cervical sympathetics were cut about the level of the cricoid cartilage high in the neck. These nerves were cut at one sitting. The cervical sympathetics were included in the cutting, since a few stray vagal fibers course through them (43); for an endeavor was made to catch and cut all vagal fibers.

Double splanchnectomy. Other cats than those studied in the preceding operation were made use of in this investigation. After the usual subcutaneous injection of atropine sulphate, the cat was etherized and laparotomized. The incision was made through the midline of the abdominal wall, in order to insure good healing and the usual aseptic precautions were observed.

After locating the left adrenal, the peritoneum directly forward of it was picked off to expose the celiac ganglion, and the splanchnic was then secured, pulled forward and cut distally from the ganglion. A second cut-

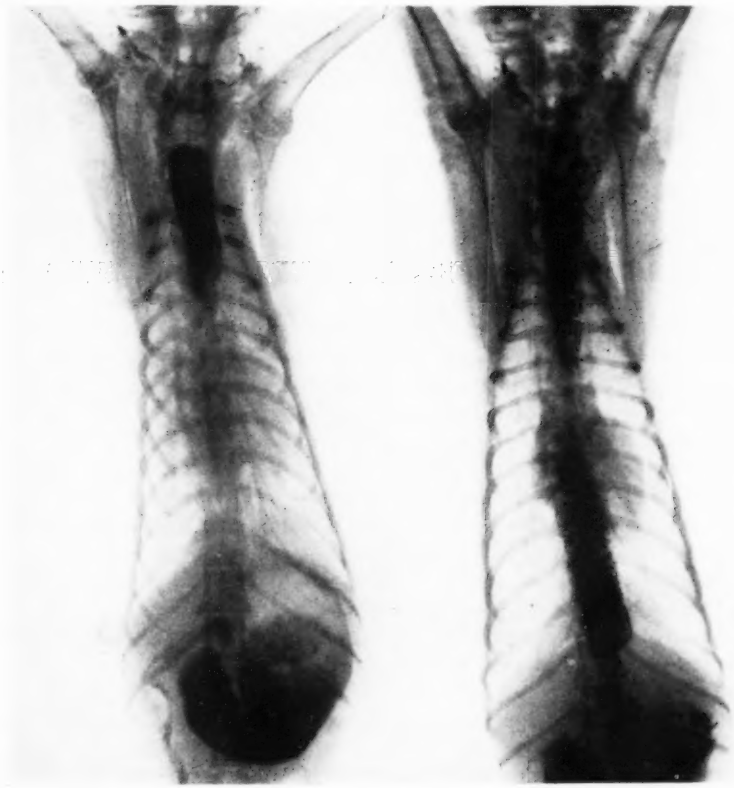

Fig. 1 Fig. 2

Fig. 1. Radiogram of doubly vagotomized cat. On deglutition of a bolus of food by a doubly vagotomized cat, the bolus remains in the striated muscle portion of the esophagus, but not in the smooth muscle portion as is evident by the empty lower end of esophagus and filled stomach; the cat had been fed about an hour before.

Fig. 2. Radiogram of doubly vagotomized cat while feeding. Note the column of food is separated by a peristaltic wave into two at upper border of heart; exposure 0.2 second.

ting served to remove the peripheral stump remaining. The presence of the liver lobes on the right side of the animal called for a slightly different procedure. This consisted of turning the liver aside and cleaning the perito-

neum and fatty tissue between the upper part of the kidney and right adrenal away. Thus one was able to secure and cut the right splanchnic nerve at the upper pole of the adrenal. This being done, the smaller splanchnic nerves on both sides of the animal were then found and severed. Whereupon the wound was closed and sewed up in layers.

Double splanchnectomy and double vagotomy. In addition to the animals already studied, a few others were prepared by the already described preliminary electrocautery of their larynx, and allowed to recover. Double splanchnectomy was then resorted to, and after a recovery from this third operation, the cats were subjected to a severing of both vagi and cervical sympathetics high in the neck.

X-ray technic. In order to insure success and obviate the necessity of the animals being held during observation, the designing of a special technic was necessary. At first I tied the fore and hind limbs of the animal separately, and then wrapped it up into a stout cloth, so as to prevent it from running away in the darkness of the observation room, in case it should become frightened by the noise of the running motors. Thus tied and lying on its side, the animal was encouraged to eat.

Many kinds of barium sulphate meals were tried; however, it was found that cats are quite fond of minced beef liver, and do not object to the barium sulphate making up the paste at all.

Another and more satisfactory device was to place an ordinary table over the Coolidge tube. Upon the table rested a movable box enclosed from all sides except the top. Two sliding rails carried the fluoroscopic screen at a level with the top of the opening of the box. For observation purposes the cat was placed in the box (this was of comfortable size), a platter of a barium liver meal was placed before the animal and it was allowed to eat. Thereupon the screen was rapidly moved into place and observations easily made.

Results. *1. Double vagotomy.* The impression current among investigators is, that animals doubly vagotomized in the neck usually are depressed for a long period and succumb rapidly, as is evident from the following quotation from Schafer (35), "The fatal result which follows double vagotomy, and which occurs within three days after section of the second vagus, no matter how long previously the first has been cut, is due not to pneumonia but to slow asphyxia caused by obstruction at the glottis from paralysis of the laryngeal muscles." However, "if precautions are taken to obviate this" by electrocautery of the larynx, "animals survive the double operation indefinitely" (35, 36). Especially one cat ("Rusty") which was thus treated preparatory to double vagotomy, survived the operations nearly nine months. This cat was exceptionally well trained, allowing itself to be handled, tied up, etc., as need be, and was not at all disturbed or frightened by the running motors of the x-ray apparatus.

In fact, it took a barium liver meal two hours after double vagotomy. Observations made with the fluoroscopic screen showed paralysis of the whole esophagus. Next day a little vomitus was found on the floor of the cage. A fluoroscopic examination of the animal revealed that the rest of the barium meal was already within the stomach, and that, before twenty-four hours had elapsed. At this time the esophagus showed paralysis in its upper part, but motility in its lower end. The usual classic symptoms characteristic of lesions of the vagus and cervical sympathetics were noted, taking their regular course, compensations following. Nevertheless, this cat manifested a sweet disposition, fed with relish and slept most of the time.

On the third day the cat was subjected to another fluoroscopic observation while feeding, and it was found that the esophagus emptied itself of the food there stagnated within half an hour.

The fourth day the animal refused food; it remained depressed for about a week, sleeping most of the time. During the first few months this animal would repeatedly relapse into such periods of depression and refuse food. These periods would last from a few days to two weeks at a time. The cat would then seek a warm place, and sleep for a while; occasionally it would get up on its feet to change for a more comfortable position, and then relapse into sleep.

Upon recovery from these periods of illness, the cat would take an interest in life and forage for food, catching wild mice and rats if given an opportunity, and would devour them. Occasionally vomiting would occur, either during a meal or after it, especially after having lapped up some liquid food. During the last months of its life, this cat was not ill at all, and would ask for food or hunt it.

However, during all this time the upper end of the esophagus was paralyzed and the lower end functional. But this motility in the lower end of the esophagus could be inhibited by frightening the cat, or by molesting it, i.e., stroking it, or pulling it by the ear while feeding. At no time was there any dilatation of the esophagus discernible.

Eight months after the double vagotomy, Doctors Carlson and Luckhardt, while observing this same cat under the fluoroscopic screen, noticed peculiar movements in the striated musculature of the esophagus, which Doctor Carlson interpreted as follows: "Besides the passive movements of the upper esophagus due clearly to the respiration and the heart beat, irregular contractions were seen. At times these contractions resembled the irregular and incoördinated contractions of the heart known as delirium cordis. At times, there appeared feeble local contractions suggestive of intestinal segmentation contractions. Feeble peristaltic and antiperistaltic waves were also seen traversing a short distance of the paralyzed part of the esophagus. None of these esophageal movements were vigorous or

coördinated enough to force the food completely out of the paralyzed portion of the esophagus. This motor automatism of the striated part of the esophagus was never seen during the first two months after double vagotomy."

A detailed study of this motility was planned, but before this could be done, tracings by the balloon method of esophageal motility of normal cats were desired.[1] In the meanwhile the animal died during the night. No apparent cause of death was found. Curiously enough, the thymus did

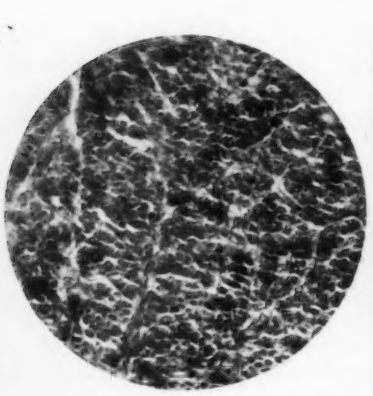

Fig. 3

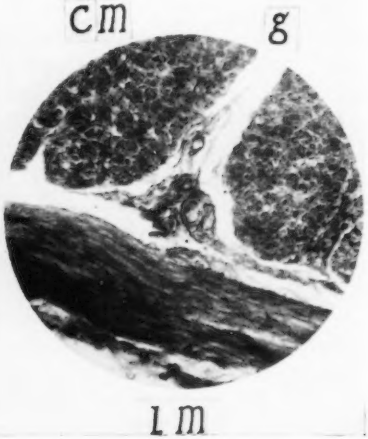

Fig. 4

Fig. 3. Cross section of the striated end of esophagus of cat nine months after double vagotomy. In comparison with a corresponding level of the esophageal musculature of a normal cat there is no atrophy.

Fig. 4. A section through the striated portion of esophagus of cat nine months after double vagotomy; *cm*, circular muscle layer; *lm*, longitudinal muscle layer; *g*, ganglion cells. Muscle fibers appear normal, and a nest of ganglion cells is discernible between the longitudinal and circular muscle layers.

not become involuted, but was remarkably massive. Moreover, the stomach was somewhat distended with air and contained some meat and few round worms. Examination of the lungs ruled out the possibility of aspiratory pneumonia as cause of death. The last x-ray observation of this cat, fortunately, was made the day before it died, and it was perceived that outside of the peculiar movements in the upper striated end of the

[1] Similar movements in the striated end of the esophagus were observed in another cat three months after double vagotomy. While tracings were being taken the animal vomited and aspirated some tenacious mucus and succumbed to asphyxia.

esophagus, this part was paralyzed; the lower smooth end manifested tertiary peristalsis as ever since nine months before.

An exploration of the neck revealed that the severed nerves on the right side of the animal, that is, the central and peripheral ends of the vagus and cervical sympathetics were in continuity through a swollen mass of tissue (neuroma). The central end of the severed nerves on the left side of the neck were found to be joined to the skeletal muscles of the neck. The peripheral stump of the nerves, however, made a junction with the left carotid artery.

An examination of stained histological preparations of these nerves manifested an invasion of white fibers of connective tissue in these nerves, both right and left. The swollen mass of tissue (neuroma) from the right nerves proved to be nothing but connective tissue. This was true also of the end in the case of the left central nerves. The left peripheral end in the region which corresponded to the sympathetic nerve, revealed a central core of nerve fibers in good condition, surrounded by white fibers of connective tissue whose axis lay in the transverse direction. The histological study of the nerves of the esophagus of this cat with respect to the vagi and cervical sympathetics showed it to be denervated to our complete satisfaction.

In this connection, may be considered the experiments of several investigators who obtained negative results in regeneration of the cut vagus. Schafer (35) concludes: "Even as long as two years and fifty days after excision of a portion of the vago-sympathetic in the cat, there is no evidence of functional regeneration in the peripheral portion of the vagus, although regenerated fibers, derived chiefly if not entirely from the sympathetic, are abundantly present in it." In other experiments done in conjunction with Feiss (34) and Tsukaguchi (43), he also got negative results; so did Langley. "Langley (21) failed to obtain evidence of recovery of function in the peripheral vagus of a cat twelve months after section. Nor was Tuckett (44) more successful in obtaining recovery of the esophageal fibers in a rabbit in which the vagus had been cut 231 days previously. Langley expresses the opinion that this lack of recovery might be a question of time 'since the distance from the point of section to the peripheral structures is considerable.'" "In no case" says Schafer (35) did we succeed in obtaining any result on stimulating the peripheral cut end although there was —as Langley also had found—abundant evidence of regenerating (i.e., down growing) fibers in the nerve, derived from the adjacent sympathetic when this was included in the section, as well as from the central end of the corresponding cut vagus nerve."

Subjecting the esophagus in turn to a similar microscopic study of suitably stained preparations, we found that the striated musculature had not degenerated but persisted. Moreover, at first it was thought that a hypertrophy of the striated muscle had taken place. However, when compared

with a similarly prepared esophagus from a normal cat, it was found that neither hypertrophy nor degeneration had occurred. Ganglion cells of Auerbach's plexus were observed between the layers of the striated muscle of the esophagus high in the neck.

Another cat which had been cauterized and whose vagi and cervical sympathetics were severed, showed no peristalsis on feeding one and a half hours after awakening from anesthetia. Nine hours later this cat fed voluntarily and peristalses (tertiary) were in evidence. The picture was identical with that of the cat first described: paralysis in the upper portion of the esophagus and motility in the lower end.

These results are typical of many other cats studied; and in cats similarly prepared but forcibly fed, the findings were also the same.

A young dog whose larynx was cauterized, and who later was doubly vagotomized in the neck, manifested great proclivity to vomiting and drinking water. It took a barium liver meal willing the first day; some of the meal passed into the stomach, the rest remaining in the esophagus. The following day this dog vomited a portion of the meal, and drank much water which was likewise vomited. It then refused to feed, felt very sick, and had great respiratory difficulties (respirations, seven per minute); it died the next day. Death was ascribed to aspiratory pneumonia.

2. Double splanchnectomy. Fluoroscopic observation of doubly splanchnectomized cats showed no difference in the movements of the column of food in the esophagus other than that of normal cats. The splanchnic nerves, autopsies showed, had been cut. It was also observed that the splanchnics readily unite together in a few weeks.

3. Double splanchnectomy and double vagotomy. A cat whose larynx was electrocauterized was doubly splanchnectomized after an elapse of two weeks. Two days later the movements of the esophagus were observed; they appeared normal. Six days after splanchnectomy, the cervical sympathetics and vagi were cut high in the neck. This animal ate freely one and a half hours after the operation. When offered food five hours later it took it willingly, but swallowed it with difficulty. At this time only two peristaltic (tertiary) waves were seen. Six hours after the operation three more peristaltic waves were observed in the lowest portion of the esophagus upon partaking of some water and a meal. Ten hours later the esophagus was emptied. Thereupon this animal fed whenever it felt so inclined. The food remained in the stomach for a long time. Death ensued the fourth day after vagotomy, presumably of peritonitis.

Discussion. Paralysis involving the whole of the esophagus follows immediately upon double vagotomy; not upon double splanchnectomy. Evidently, the vagi are of more importance in the control of this organ than the splanchnics. It is a common occurrence that organs dependent upon an extrinsic nerve supply cease activity when the nerves are removed.

Accordingly, the musculature of the blood vessels is also paralyzed, when vasomotor nerves are cut, as in pithing of the spinal cord. However, after a time, the musculature of the blood vessels assumes its specific activity: smooth muscle generally possesses an automatism of its own. This automatism may be so dominated by extrinsic nerves that it does not express itself; it is as if it were subdued. When, however, this influence of extrinsic nerves is removed, the automatism can then exercise its powers. It is for this reason that, in cases following vagotomy, the esophagus in that part where it is composed of smooth musculature and Auerbach's plexus, can act by itself. The relation of the nerves to the muscles of the blood vessels, and the relation of the nerves to the musculature of the gut (including the esophagus) is not so close; especially, if one views it from the hypothesis: "that the visceral nerves are complex association paths connecting cerebrospinal and visceral reflex centers, rather than relatively simple nerves like the skeletal efferents" as proposed by Carlson (7).

Muscle, whether striated or smooth, possesses independent irritability and contractility. The mechanical distention of the esophageal tube in parts where it is composed of smooth muscle, is an adequate stimulus (as in the rest of the gut, or in hollow viscera), causing it to respond by its specific activity, i.e., contraction. This contraction is coördinated. Inasmuch as there are well-described (15) and illustrated (12, 15, 33) ganglion cells, some of which belong to the Auerbach's plexus in the esophagus of man (15) and other animals (12, 33), which in the case of man (15) extend downward from as high a point as three centimeters from the larynx, could not the Auerbach's plexus act in this capacity of a coördinating system? Our histological preparations of the esophagus of cats clearly showed that these ganglia are present even in the part of the esophagus composed of striated musculature. The splanchnics also supply the smooth region of the esophagus; we can readily see, then, that the smooth musculature still has a nerve supply even though the vagi are cut. The splanchnic nerve supply might explain why we can get inhibition of tertiary peristalsis upon frightening or molesting a feeding animal whose vagi were cut, but whose splachnics were intact. Openchowski (27, 28) makes a claim, which is confirmed by Carlson's investigation (8), viz., that there are sympathetic efferents going to the esophagus through an aortic and gastric plexus. We see, then, that to denervate the esophagus completely is practically impossible. Thus the motility of the esophagus observed after section of vagi and splanchnics may be accounted for by the other sympathetic efferents or by the intrinsic plexus; some might claim by the muscle itself (myogenic theory). At any rate, the smooth muscle portion of the esophagus shows, as do viscera composed of smooth muscle, a considerable degree of automatism.

But what of the striated portion of the esophagus? The kind of motility

observed in the striated portion of the esophagus, must be subjected to a graphic study before anything can be said about it with confidence. Prakken (30) studied strips from the striated portion of the esophagus of cats; he found the strips to contract rhythmically in warm oxygenated Ringer's solution. Carlson thinks "such a mechanism would not serve deglutition hence it is not physiological" (7). The impression gathered from observing the motility of the striated portion of a vagotomized esophagus of cat, was that of incoördinated rapid irregular contractions with feeble peristaltic waves moving forward and backward (antiperistaltic). Could it not be, granted that there is a specific motility of some kind or other not due to heart beat, pulse, respiratory movements, etc., that there is a return of some primitive activity of striated muscle which was lost in the embryonic differentiation? Or, could it be a restoration of tertiary peristalsis, wherein the muscle and Auerbach's plexus act as a unit? Or, might this activity be due to regeneration of a few vagal fibres? Or, perhaps, might not the sympathetic motor fibres grow into this region (striated portion of esophagus), in much the same manner as a nerve that does not supply a certain muscle will, in some cases, sprout a branch to it, when the nerve that normally innervates the muscle has been removed?

The other curious observation was that the striated muscle in the upper portion of a doubly vagotomized esophagus did not degenerate, nor become atonic. The sympathetics or the local ganglia, or both, may act, perhaps, as trophic centers for it in the absence of the vagi.

The swallowing of a morsel of the barium-liver meal was equivalent to the projection of the bolus of food into the proximal end of the esophagus, where it would remain. Succeeding swallows would accumulate the food here, and the column of food thus massed would by subsequent deglutitions and gravity be pushed downward. About the upper border of the heart peristaltic (tertiary) waves would nip off a segment; additional waves would appear and subdivide, at times, the segment into smaller masses, which would then be swept down past the cardia without interference into the stomach. The tertiary peristaltic waves are, perhaps, elicited by the distention of the part composed of smooth musculature by the food. These waves appeared only when food reached this part. Occasionally, an antiperistaltic wave or two would bring back a little of the food that had already passed the cardia. This would occur when the stomach was already fairly well distended with food.

SUMMARY

1. The immediate effect of double vagotomy of cats high in the neck, is a paralysis of the whole esophagus more or less complete, involving the stomach as well.

2. An early (9 to 24 hours) recovery occurs in the lower end of the esophagus, whereas the upper end remains paralyzed.

3. This difference of recovery of the esophagus corresponds to the different composition of esophageal musculature, viz., motility in the smooth portion (lower end), and paralysis in the striated portion (upper end). These observations confirm similar findings of Cannon.

4. The cat has a proclivity to vomit in the beginning, especially on lapping liquids. This is true of dogs as well.

5. Tertiary peristalsis of the esophagus of doubly vagotomized cats can be inhibited by fright or molestation of the animal.

6. The food swallowed by doubly vagotomized cats lodges in the upper part of the esophagus forming an elongated column.

7. This food column is moved downward by subsequent swallows and also by the gravity of the barium liver meal.

8. The column of food in the esophagus of doubly vagotomized cats, is pinched off by a peristaltic wave about the level of the upper border of the heart, corresponding to the smooth musculature.

9. The striated portion of the esophagus of cat regains some motility within three months or less after double vagotomy.

10. The striated muscle portion of the esophagus of cat does not degenerate, nor does this part of the esophagus dilate even as late as nine months after double vagotomy.

11. Double splanchnectomy does not affect the motility of the esophagus of cat.

I wish to express my deep appreciation and gratitude to Dr. A. J. Carlson for his kind inspiration, invaluable suggestions and criticisms rendered during the course of this investigation.

BIBLIOGRAPHY

(1) Bercovitz and Rogers: This Journal, 1921, lv, 323.
(2) Bernard: Compt. Rend. Soc. Biol., 1849, i, 14.
(3) Bottazzi: Journ. Physiol., 1899, xxv, 157.
(4) Bottazzi: Pflüger's Arch., 1906, cxiii, 136.
(5) Cannon: This Journal, 1907, xix, 436.
(6) Cannon: The mechanical factors of digestion. London, 1911.
(7) Carlson, Boyd and Pearcy: This Journal, 1922, lxi, 14.
(8) Carlson, Boyd and Pearcy: Arch. Int. Med., 1922, xxx, 409.
(9) Carlson and Litt: This Journal, 1923, lxv, 534.
(10) Carlson and Luckhardt: This Journal, 1921, lvii, 299.
(11) Chauveau: Journ. de l. Physiol., 1862, v, 337.
(12) DeWitt: Journ. Comp. Neurol., 1900, x, 382.
(13) Doyon: Arch. d. Physiol., 1895, vii, 374.
(14) Goltz: Pflüger's Arch., 1872, vi, 616.
(15) Greving: In Muller's "Die Lebensnerven," Berlin, 1924, p. 230.

(16) Hopf: Zeitschr. f. Biol., 1911, lv, 409.
(17) Howell: Physiol. Rev., 1925, v, 161.
(18) Krehl: Arch. f. Physiol., 1892, Suppl, 278.
(19) Kronecker and Luscher: Arch. Ital. de Biol., 1896, xxvi, 308.
(20) Langley: Journ. Physiol., 1889, xxiii, 807.
(21) Langley: Journ. Physiol., 1895, xviii, 280.
(22) Langley: Ergebn. d. Physiol., 1903, ii, 818.
(23) May: Journ. Physiol., 1904, xxxi, 260.
(24) Meltzer: This Journal, 1899, ii, 270.
(25) Meltzer and Auer: Brit. Med. Journ., 1906, ii, 1806.
(26) Morat: Arch. d. Physiol., 1893, v, 142.
(27) Openchowski: Zentralbl. f. Physiol., 1889, iii, 1.
(28) Openchowski: Arch. f. Physiol., 1889, 549.
(29) Patterson: This Journal, 1921, lvii, 169.
(30) Prakken: Arch. neerd. d. Physiol., 1920, iv, 487.
(31) Ravitsch: Arch. f. Anat. u. Physiol., 1861, 770.
(32) Reid: Edinburgh Med. Surg. Journ., 1839, li, 329, 274.
(33) Sabussow: Anat. Anz., 1913, xliv, 64.
(34) Schafer and Feiss: Quart. Journ. Exper. Physiol., 1916, ix, 329.
(35) Schafer: Quart. Journ. Exper. Physiol., 1919, xii, 231.
(36) Schafer: Quart. Journ. Exper. Physiol., 1920, xii, 367.
(37) Schiff: Physiol. d. l. Digestion, 1867, ii, 377.
(38) Sinnhuber: Zeitschr. f. klin. med., 1903, 1, 102.
(39) Smith: This Journal, 1918, xlvi, 232.
(40) Spandolini: Arch. d. fisiol., 1916, xv, 229.
(41) Starck: Münch. Med. Wochenschr., 1904, li, 1512.
(42) Steinach: Pflüger's Arch., 1898, lxxi, 523.
(43) Tsukaguchi: Quart. Journ. Exper. Physiol., 1916, ix, 281.
(44) Tuckett: Journ. Physiol., 1896, xix, 297.
(45) Veach: This Journal, 1925, lxxi, 229.
(46) Veach: Journ. Physiol., 1925, lx, 457.
(47) Waters: Journ. Physiol., 1885, vi, 460.

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A MODEL TO SIMULATE THE MECHANISM OF EMPTYING OF THE GALL BLADDER

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In all of the enormous amount of discussion of the mechanism of emptying of the gall bladder which has been going on in recent years, attention has been focussed almost solely on the question of whether or not there is a contractile action of the musculature of the wall resembling the peristalsis of the intestine. The development of cholecystography in this laboratory by Graham, Cole and Copher (1) has lent additional interest to this question because a method was thereby created which, by permitting the roentgenological visualization of the gall bladder in the normal subject, seemed to provide an opportunity to see any contractile waves if any should occur in a manner analogous to those seen in the stomach and intestine after a barium meal. Despite the fact, however, that the cholecystographic shadow of the gall bladder disappears after varying periods of hours, depending on conditions present, no one has so far reported observing by this method anything resembling a peristaltic action of the gall bladder. Moreover, Sweet (2) has even questioned whether the bile of the gall bladder ever leaves the organ by way of the cystic duct because of the anatomic difficulties such as the presence of the Heisterian valves, the acute angle of the cystic duct, the relatively weak musculature of the gall bladder, etc.

The purpose of this brief article is to show that in all the discussion one important simple mechanical principle has usually been overlooked, namely, elasticity, although Boyden (3) has given prominence to this principle. The gall bladder, like the other hollow viscera of the body, is elastic. It can be easily distended and it has a very appreciable elastic recoil when the inside pressure is suddenly relieved. Experimental work which is briefly summarized in this article shows conclusively that this simple mechanical principle of elastic contraction is a very important, and perhaps the most important, factor in emptying the gall bladder. When the sphincter of Oddi[2] is closed, the gall bladder is being constantly distended

[1] This work was aided by a grant from Edward Mallinckrodt, Jr.

[2] The term "sphincter of Oddi" has been used in this article to represent the mechanism of closure of the ampullar end of the common duct, but in a later paper, now in press, evidence will be given to show that tonus of the duodenal musculature is perhaps even more important than the so-called "sphincter of Oddi."

by the reception of bile. When, however, the intestinal end of the duct is suddenly opened there is a sudden diminution of pressure within the gall bladder. Elastic recoil then occurs and bile is squirted through the cystic duct with considerable force. This discharge of bile from the gall bladder will continue until the pressures within the gall bladder and within the common duct have come to an equilibrium. Obviously, therefore, the gall bladder never empties itself completely at one time as it would if a peristaltic contraction occurred. Its contents, however, will gradually undergo dilution and change if a succession of openings and closings of the intestinal end of the duct occurs, because, after every closure of the sphincter,

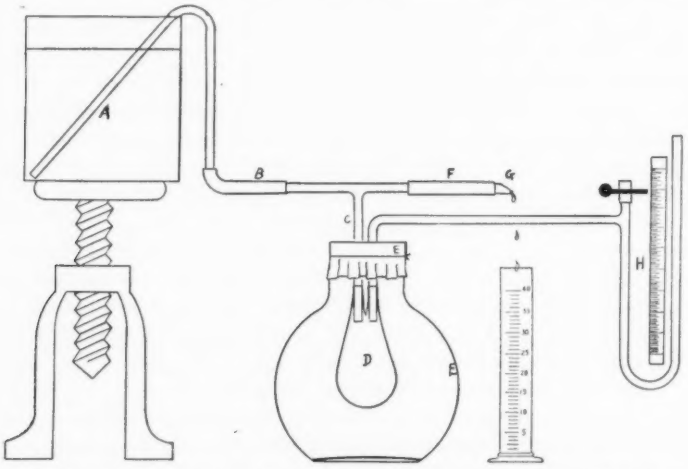

Fig. 1. Model to demonstrate the mechanism of emptying of the gall bladder; *A*, water in large flask; *B*, hepatic duct; *C*, cystic duct; *D*, gall bladder; *E*, abdominal wall; *F*, common duct; *G*, Oddi's sphincter; *H*, manometer to record changes of pressure inside gall bladder.

more liver bile will pour into the partially emptied gall bladder which will again discharge some of its contents as soon as the sphincter reopens. In this way the gall bladder will be gradually washed out by the repeated opening and closing of the end of the common duct.

There is perhaps also another mechanical principle involved which has hitherto escaped notice. This is the principle of the filter pump which is well known to chemists. If, with the sphincter of Oddi open, a steady stream of bile passes down the common duct past the cystic duct some of the contents of the gall bladder will be sucked out through the cyst duct, provided that there is no obstruction to their passage through the common duct.

To illustrate these points a simple model was devised which made use of a rubber balloon to represent the gall bladder. A real gall bladder, however, was shown to act in the model in exactly the same manner as the rubber balloon. A description of the model follows:

A small rubber balloon was connected to two glass tubes and suspended in a flask which was covered with a thick rubber sheet through which the tubes passed. The reason for suspending the balloon in a flask was to avoid the effects on it of outside pressure and to simulate as closely as possible normal conditions, the rubber sheet acting as the abdominal wall. One of the glass tubes was connected with a small manometer with which to register the pressure within the gall bladder, and the other tube was a T-tube, one end of which was connected with a siphon from a beaker or with the water faucet, the other end being connected with a glass tube drawn out to a small caliber at one end to represent the opening of the common duct at the ampulla. The continuous tube, therefore, from the beaker or water faucet to the small end represented the hepatic and common ducts and the right angled branch represented the cystic duct. If the gall bladder is filled with water containing a dye through the manometer it will be easy to determine when the contents of the gall bladder are being discharged.

If now a stream of water is allowed to pass slowly down the common duct out of the ampullar end, very little change occurs in the gall bladder itself. If, however, the ampullar end is closed with the finger to represent the closing of the sphincter of Oddi the inside pressure of the gall bladder is seen to rise, as recorded on the manometer, and the gall bladder becomes more and more distended. If the finger is suddenly removed from the end of the tube, imitating the opening of the sphincter of Oddi, a jet of colored water is seen to pass down the cystic duct into the common duct and out of the ampullar end. The discharge of the colored contents of the gall bladder continues until the pressure of the inside of the gall bladder comes to an equilibrium with that of the common duct. But it can be repeated again by successive closing and opening of the ampullar end of the common duct. It is thus evident that the artificial gall bladder, and most probably the gall bladder *in situ*, never empties itself at once. In fact, the only way by which the artificial gall bladder can get rid of its colored contents is by a gradual washing out. For example, when an artificial gall bladder containing 5 cc. of 1 per cent phenoltetrachlorphthalein was used in the model, 280 cc. of water from the artificial hepatic duct were required before the contents of the gall bladder became colorless; and, in order to accomplish this, the mouth (ampullar end) of the tube was opened and closed by the finger at the rate of thirty times per minute.

These simple observations seem to have a fundamental bearing on many questions, and the theory based upon them, namely, that the emptying of

the gall bladder is largely a question of the elastic recoil of a distended viscus seems to find confirmation in various other experimental observations. For example, when a fistula of the common duct was made in a dog below the junction with the cystic duct the injection into the duodenum of various substances which are known to cause an outpouring of bile had no influence in making the thick dark bile of the gall bladder appear. The bile which came through the fistula was always the thin yellow bile of the liver. The substances which were used were various kinds of food, Witte's peptone and magnesium sulphate. In not a single instance did the thick bile of the gall bladder appear. This experiment lends support to the idea that in order to have the gall bladder empty an elastic recoil from over-distention is necessary; for obviously, under the conditions of the experiment, the pressure in the gall bladder could not be great enough to permit its overdistention. Moreover, if the theory as derived from the model is correct, a single application of a substance to the duodenum cannot be expected to empty the gall bladder, regardless of whether the substance used is magnesium sulphate, as suggested by Lyon, or a more powerful stimulant to the flow of bile such as fat, as recently suggested by Boyden (3) and by Whitaker (4). In fact, a serious doubt on the possibility of an efficient drainage of the gall bladder by the Meltzer-Lyon method arises.

A more elaborate discussion of other questions involved in the mechanism of the emptying of the gall bladder will be discussed subsequently in another paper.

CONCLUSION

1. The elasticity of the gall bladder wall and the inside pressure of the lumen of the gall bladder are perhaps the most important factors in the flow of the gall bladder bile. Increased intraabdominal pressure is an important auxiliary factor.

2. The contents of the gall bladder are renewed by the opening and closing of the sphincter of Oddi.

3. The gall bladder never empties itself at one time after taking food.

BIBLIOGRAPHY

(1) Graham, E. A., W. H. Cole and G. H. Copher. Journ. Amer. Med. Assoc. 1925, lxxxiv, 1175.
(2) Sweet, J. E. Internat. Clinics, 1924, i, 187.
(3) Boyden, E. A. Anat. Record, 1925, xxx, 333.
(4) Whitaker, L. R. and G. Milliken. Surg., Gynec. and Obstet., 1925, xl, 17; 646.

STUDIES IN THE PHYSIOLOGY OF VITAMINS

IV. Vitamin B in Relation to Gastric Motility[1]

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The physiological basis for the anorexia (Karr, 1920; Cowgill, 1921) produced experimentally in dogs by feeding diets adequate except with respect to vitamin B presents a problem worthy of study. The symptoms of gastro-intestinal disturbances shown by dogs suffering from a lack of the anti-neuritic factor suggest that the loss of appetite in such animals is associated with partial or complete loss of the so-called "hunger contractions" of the stomach (Carlson, 1916). That these alimentary disturbances are not confined to the dog, but are characteristic of vitamin B deficiency in other species as well, is attested by the work of numerous investigators using pigeons for study, of students of beriberi in man (Vedder, 1913), and of many workers who have used rats in their investigations (Gross, 1924; Cramer et al., 1921, 1922, 1923). Any study, therefore, of the relation between the anti-neuritic vitamin and gastro-intestinal functions should possess value not only for elucidation of the rôle of this accessory factor in nutrition, but for the general physiology of the alimentary tract as well.

Experimental. The experiments reported in this paper were planned with the view of determining, if possible, whether the progressive loss of appetite characteristic of vitamin B deficiency in dogs is concomitant with a change in the character or a disappearance of the hunger contractions of the empty stomach.

Four dogs having permanent gastric fistulae were fed artificial diets similar to those employed in our earlier vitamin B studies and described elsewhere (Cowgill, 1921; 1923). The basal diets were free from or extremely low in content of the anti-neuritic vitamin.

[1] A preliminary report of these experiments was given before the annual meeting of the Section on Gastroenterology and Proctology of the American Medical Association at Chicago, Ill., June, 1924.

The expenses of this investigation were defrayed in part by a grant from the Russell H. Chittenden Fund for Research in Physiological Chemistry.

The motility of the empty stomach was recorded by the balloon method used so extensively by Carlson and his collaborators. In some of our earlier trials tracings were made on smoked paper. This method of obtaining records proved very unsatisfactory chiefly because the cylinders usually had to be changed in the middle of a series of gastric contractions, and a perfectly complete record of the series therefore was not obtained. The desire for continuous unbroken records extending over any experimental period led us finally to perfect a system whereby the contractions were recorded in ink on an "endless" roll of white paper. The paper was moved by a Cambridge kymograph apparatus. The pen points were made by drawing out glass tubing of small diameter. By means of different colored inks and by having the paper moved over two cylinders in such a way as to present both sides for recording purposes it became possible to secure tracings of the contractions of *three* dogs simultaneously, two tracings being made on one side of the paper and one on the other side. Some of the records thus obtained covered as long a period as nine hours.

The first fistula animal was prepared by using a cannula such as Pawlow (1902a) has employed. The other three animals had "valves" constructed in accordance with the scheme employed in the Franck operation or its modifications.[2] As Carlson has pointed out, such fistulae allow very little if any leakage of gastric contents, and if the animals are used in experiments twice a week the opening does not close.

Each animal was trained to lie quietly on the table with a balloon in the stomach while records were being made. The dog would usually go to sleep if the head was covered with a cloth. Every precaution in the way of making the conditions comfortable was taken. The dog boards were padded and restraining ropes for the legs were not used. Instead, wide strips of cloth encircled the animal and the board, one cloth around the neck, another just below the axillae, and the third at the hip joints. The animal was therefore free to turn considerably and to place itself in the most comfortable position consistent with the recording of contractions. One of the dogs—used by D. and P. in the laboratory at Cornell—became so well trained that it would go to sleep with a balloon in its stomach while lying on a table with no restraining cords or cloths whatever.

We have described the technic employed in more detail perhaps than may seem necessary *1*, because our system of recording gastric contractions possessed some new features and *2*, because the behavior of our experimental animals was most favorable for this type of physiological research.

Gastric records were obtained usually during two to four or more hours, beginning at about 10 a.m. The dogs were offered their daily ration immediately after records had been taken. When the animal had lost the desire to eat, either partially or completely, while subsisting on the vitamin

[2] The animal used at Cornell was operated on by Dr. P. Coryllos.

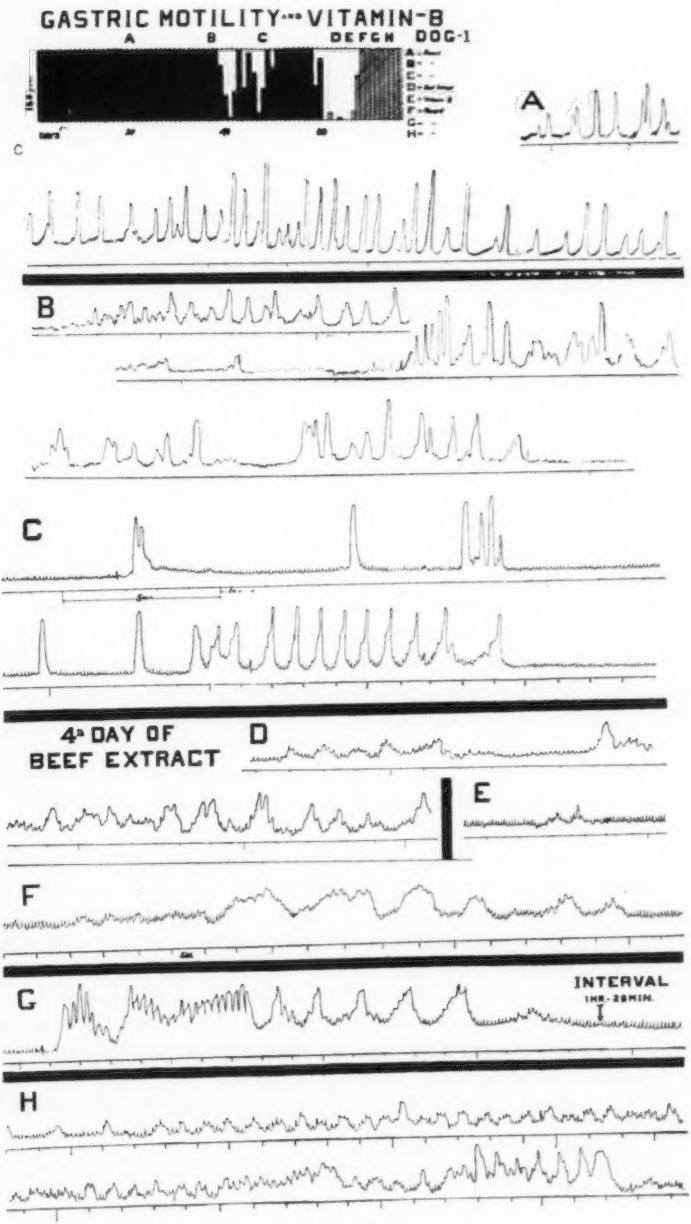
GASTRIC MOTILITY··· VITAMIN-B
A B C DEFGH DOG-1
A
B
C
4ª DAY OF BEEF EXTRACT
D
E
F
G
INTERVAL 1HR.-28MIN.
H

Fig. 1

B-free food, a control experiment was performed in which four or more large daily administrations of commercial beef extract were made. This material is practically free from vitamin B (Damon, 1922) and numerous experiments made previously (Cowgill, 1922; Cowgill, Deuel and Smith, 1925) had demonstrated that it does not restore the appetite to avitaminotic animals. When given in the amounts used in these experiments beef extract promotes the flow of gastric juice on normal animals (Pawlow, 1902b; Ivy and McIlvain, 1923). When the effect, if any, of beef extract administration on gastric motility had been observed and this treatment had not corrected the anorexia, suitable amounts of a vitamin preparation were given and the characteristic restoration of appetite followed. The sources of vitamin used in these experiments were "Yeast Vitamin Powder" (Harris)[3] and "Vitavose."[4] Proper dosages were arrived at by consideration of data from numerous quantitative experiments performed previously (Cowgill, Deuel and Smith, 1925).

RESULTS. The results obtained with all of the animals agree so well that the main points to be set forth can be illustrated quite satisfactorily by the graphic records of dog I which are presented in this paper. The composite figure has been prepared with a food intake chart as an index to the individual gastric records presented. Corresponding letters show on what day of the experimental feeding period the gastric record was made. For example, record A was made on the 20th day of the experiment. The black ordinate columns represent the amount of food eaten by the animal.

Record A (fig. 1) shows that after 20 days on the diet without vitamin B the gastric motility is not markedly influenced. The time occupied by the entire series of contractions in this case was approximately 55 minutes, individual contractions occurring usually at one minute intervals. This record is not appreciably different from those obtained previously and those obtained several months later at a time when the dog was receiving liberal amounts of vitamin B daily.

On the 36th day, which was 2 days before the first sign of loss of appetite appeared, record B was taken. Instead of a long series of contractions of great amplitude, as exhibited in record A, there appear instead several short series, each having relatively few vigorous contractions. This tendency toward a separation of the long series into several short ones is further exemplified in record C, which was made when the animal had shown anorexia of moderate degree over a period of about one week. The tracing C shows only a few contractions and no rhythmic tonus whatever. When the appetite failed completely on the 61st day four large daily doses of beef extract were administered. Record D was secured on the 4th day of beef

[3] From the Harris Laboratories, Tuckahoe, N. Y.

[4] From the Ward Baking Co., New York. See Charles Hoffman, Journ. Ind. Eng. Chem., 1925, xvii, 498.

extract treatment. It is quite evident that a marked loss of gastric tonicity had occurred. It will be noted that the administration of beef extract did not cure the anorexia. Tracing E was made just before vitamin B was administered to the animal. Although the record covered a period of several hours, the only suggestion of any contraction whatever was that shown here. That this result was not an artifact is borne out by the high

TABLE 1

ANIMAL AND DAY GASTRIC RECORD WAS OBTAINED	TIME	DURATION OF PERIODS	
		Of contractions	Of quiescence
		minutes	*minutes*
Dog. I. Record obtained on 85th day of subsistence on the "complete" diet	10:40–11:00	20	
	11:00–12:19		79
	12:19–12:41	22	
	12:41– 1:57		76
	1:57– 2:19	22	
	2:19– 3:34		75
	3:34– 3:52	18	
Dog II. 86th day on the "complete" diet	10:20–10:53	33 (vigorous)	130
	10:53– 1:03		
	1:03– 1:13	10 (slight)	
	1:13– 3:07		114
	3:07– 3:24	17 (slight)	
Dog II. 87th day on the "complete" diet	9:0 –10:29	39	
	10:29– 1:07		158
	1:07– 1:40	33	
Dog II. 94th day on "complete" diet	3:37– 4:21	44 (vigorous)	
	4:21– 7:37		196
	7:37– 8:23	46 (vigorous tetanus)	
Dog II. 135th day on the "complete" diet	11:17–11:40	23 (vigorous)	
	11:40– 2:23		163
	2:23– 2:45	22 (vigorous)	

degree of sensitivity of the balloon and manometer shown here to be responsive to changes of intra-abdominal pressure associated with respiratory movements. It should be remarked that the avitaminotic condition of the animal at the time record E was made was very serious indeed.

Tracing F was made on the first day of complete recovery of appetite following vitamin B administration. In this record the contractions which occurred were quite definitely of the tetanic type; a slight rhythmic tonus

also appeared. In tracing G is shown an improvement in the ability to relax quickly, and the interval of quiescence between groups of contractions has shortened to 1 hour and 20 minutes. The record made after this period of inactivity was almost identical with that shown in G.

At H is given part of the record obtained on the 8th day after vitamin therapy was instituted. The dog's general condition had improved remarkably and all food was being consumed immediately when offered. Record H shows good rhythmic tonus, with increasing intensity of contraction, and the gradual approach to vigorous hunger contractions.

Dog I was then given large amounts of vitamin B daily over a period of more than 2 months, and gastric records were obtained from time to time. These later records illustrate in clear-cut fashion the cycles of contractions which have been described by Carlson and his associates and undoubtedly represent the best that the stomach of this animal could do under our experimental conditions.

Table 1 was prepared from the records of dogs I and II when these animals had subsisted on the standard diet made complete by liberal amounts of vitamin B administered daily and illustrates the cycles of hunger contractions.

It should be remarked that the experiment with dog I, just described in detail, was a repetition of another one carried out many months before which yielded the same results. The conclusions, therefore, may be regarded as having been confirmed on the *same* animal as well as on other animals.

The results obtained with dog II are interesting because they show that constipation is a factor in the development of loss of desire to eat. Twice this animal lost its appetite almost entirely while subsisting on the vitamin-free food. Upon receiving large doses of beef extract, resulting in the passage of four semi-diarrheal stools, the desire to eat was restored. Eventually, however, a loss of appetite occurred concomitant with other more general symptoms of vitamin B deficiency described elsewhere (Cowgill, 1921). From the symptoms presented at the time beef extract administrations had proved ineffective, it was quite evident that vitamin administration was necessary. The records of stomach motility obtained at this time revealed a condition of gastric atony. Vitamin therapy brought about a prompt restoration of the appetite, evacuation of the bowel, and a general improvement in the picture of gastric motility. Large daily doses of vitamin were then given over a period of approximately 2 months, and gastric records were obtained from time to time as in the case of dog I. These later records show the characteristic supposedly normal cycle of contractions. Data from these records have been incorporated in table 1.

In previous experiments with other animals it had been learned that the appetite cannot be restored to dogs in an advanced stage of vitamin B

deficiency merely by evacuating the bowel with castor oil treatment. In spite of the forced elimination of fecal material at such a time the characteristic nervous and muscular symptoms have developed within a few days and improvement and cure have been brought about only by administration of the vitamin. We are inclined to the view that in the majority of cases failure of our animals to defecate can be ascribed to failure to eat as well as to any other cause.

Dog III was a much younger animal than the others of the series. Its earlier records were characterized by unusually long periods of contractions —approximately 55 minutes—and relatively short quiescent periods. Like other young animals we have fed on vitamin B-free rations, it ate the food much longer than the others before showing anorexia. For over 100 days this animal continued to eat all of the ration offered. The gastric records obtained from time to time, however, showed changes similar to those already described for dog I. From the 108th to the 111th days a condition of gastric atony was approximated. The dog was found dead the morning of the 112th day. In one of our earlier papers (Cowgill, 1921) the sudden death of avitaminotic animals presumably due to heart failure during a convulsion is described. It is our belief that the death of dog III was due to such a cause.

Dog IV was used for study by two of us (Deuel and Plummer) in the laboratory at Cornell University Medical College. This animal is of particular interest because it developed the severe nervous and muscular symptoms characteristic of an advanced stage of vitamin B deficiency and its condition was relieved by the administration of the missing dietary factor. The average period of activity and quiescence of the stomach of dog IV between the 1st and 54th day of the vitamin B-free regimen shows a mean period of 65 minutes of activity followed by one of 32 minutes in which no gastric motility or only slight tonus was noted. On the 3 hour basis on which practically all the experiments were carried out, this amounts to 121 minutes of activity and 59 minutes of inactivity. It will be noted from table 2 that, although the actual duration of these periods varies from day to day, as is the case with an animal on a diet including vitamin B, the hunger contractions are of a normal frequency and duration as well as the usual intensity after the desire for food has been practically lost (the 8th day) and when the first symptom of polyneuritis, namely, the spasticity, has appeared. The records of gastric motility obtained show that on each of the three days when the symptoms were severe no sign of gastric contractions was obtained over a period of 3 hours. On the recovery from polyneuritis following the administration of vitamin B, as is evident from consideration of columns 4, 5, 11 and 12, there is a slight restoration of the tonus of the stomach before the appetite returned, although in general the reappearance of the appetite was concomitant with the restoration of the normal frequency and intensity of the stomach contractions.

TABLE 2

DAY ON DIET	TOTAL LENGTH OF RECORD	ACTIVE PERIOD	QUIESCENT PERIOD	HEIGHT OF CONTRACTIONS				TYPE OF CONTRACTION	FOOD EATEN OF 220 GRAMS OFFERED	REMARKS
				4–6	6–8	8–10	Over 10			
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
	min.	*min.*	*min.*	*cm.*	*cm.*	*cm.*	*cm.*		*cm.*	
54	180	60	120	37	13	10	36*	III	220	
57	180	150	30	45	20	13	3	I and III	220	
58	180	139	41	37	16	19	2	I	220	Tonus throughout
63	180	124	56	52	26	13	3	I	148	
64	180	123	57	21	21	14	6	I	220	
68	180	76	104	13	4	3	0	I	185	
69	180	135	45	21	8	2	0	I	95	Tonus throughout
71	180	110	70	13	8	9	1	II	80	Tetanus for 6 minutes
72	180	126	54	32	25	1	1	I	119	
73	180	130	50	65	25	11	0	II and III	90	
75	180	100	80	43	16	3	0	I and II	0	Tonus throughout
76	180	65	115	42	19	3	0	I	120	Tonus pronounced throughout
78	180	98	82	27	5	5	0	I and II	45	Tetanus for 5 minutes
79	180	110	70	37	23	15	5	I	0	
80	180	145	35	39	8	0	0	I	50	Spasticity first noticeable
82	180	36	144	19	12	6	1	I	0	
83	180	29	151	10	6	6	0	I	0	One period of activity with long quiescent period before and after
85	180	12	168	3	2	0	0	III	0	Polyneuritic symptoms
86	180	0	180	0	0	0	0		0	Slightly increased tonus for 5 minutes Polyneuritic, with spasticity and slight involvement of fore legs
87	180	0	180	0	0	0	0		0	Dog very sick. Vomited bile-colored fluid while records were being made. 10 gm. Harris powder given at conclusion of tracings

88	180	0	180	0	0	0	0		0	Dog much relieved
89	180	17	163	7	0	0	0		0	Slight tonicity onset. 7 gm. Harris powder given
90	180	45	135	19	6	0	0	III	45	Tetanus for 9 minutes. Dog much improved but spasticity still present
91	180	121	59	27	16	3	0	II	175	
92	180	154	26	163	39	7	0	II	220	Tetanus for 16 minutes.
93	180	158	22	162	63	5	0	II and III	220	

* Many contractions 16 to 18 cm. high.

An attempt has been made to convey some idea of the amplitude of the individual contractions by the data given in columns 6, 7, 8 and 9. It is recognized that measurements of the height of contractions are at best only roughly approximate because of possible differences due to variations in the pressures within the balloon from day to day. Even with this qualification in mind, mere inspection of the figures cannot fail to impress one with the diminution in amplitude occurring just before the onset of severe polyneuritis and the rapid improvement in gastric motility following vitamin therapy. The data presented here were taken from records made on smoked paper and quite definitely confirm the findings shown graphically in figure 1.

Discussion. Analysis of all of the data reveals the fact that in animals subsisting on diets adequate except with respect to vitamin B two fairly well-defined conditions are to be distinguished: 1, that in which the desire to eat is partially lost but in which no symptoms of severe vitamin deficiency, such as vomiting or clonic spasms, occur; and 2, that condition in which a loss of appetite is associated with the symptoms just mentioned. Perhaps these may more aptly be called mild and severe cases of vitamin B deficiency.

The partial loss of appetite that occurs in mild cases of this deficiency does not appear to be associated with any very remarkable changes in the hunger contractions of the stomach. How much a depressed motility of the intestines—exhibited by constipation—figures as a cause of the anorexia in the mild cases, can only be guessed at the present time. That it does play a rôle, as claimed by Gross(1924) from his observations of rats, seems supported by our studies on dogs. We have planned experiments designed to elucidate the relation of vitamin B to intestinal motility in dogs and hope to perform them soon. With respect to the severe cases it may be said that whenever the hunger contractions definitely disappeared anorexia developed; on the other hand, the return of appetite following vitamin B therapy was always more prompt than the return of the gastric motility to anything like the normal condition.

Cramer, Drew and Mottram (1921, 1922) and Cramer (1923) believe that vitamin B functions in a peculiarly specific manner to promote the activity of the lymphoid tissues, and attribute the alimentary disturbances characteristic of B avitaminosis to altered function of this tissue. We are not convinced by the evidence which they submit. Even though the pathological findings of Cramer and his associates with respect to the lymph nodes located along the alimentary canal should be correct, they would not prove that the primary organic factor in B avitaminosis is failure of the lymphoid tissue to function in normal fashion. McCarrison (1921) and Beznak (1923) have shown hypertrophy of the adrenal glands to be characteristic of vitamin B deficiency and in harmony with Cramer's

viewpoint one would be equally justified in attributing this syndrome to adrenal dysfunction.

When one considers the nervous and muscular symptoms, as well as evidence of alimentary dysfunction exhibited by animals of different species suffering from vitamin B deficiency, and gives due weight to those factors which seem to determine how much vitamin B a given organism requires for physiological well-being (Cowgill, Smith and Beard, 1925), it becomes very difficult to accept the hypothesis presented by Cramer and his associates. However, so many factors seem to be involved in the problem as to the real physiological rôle of vitamin B that, for the present at least, all the ideas advanced can be regarded only as provisional working hypotheses. The recent findings of Farmer and Redenbaugh (1925) suggest that this dietary factor may be a precursor of certain alimentary enzymes but even in this work no control against the effect of starvation alone on the alimentary organs appears to have been made.

Quite apart from the question as to *how* vitamin B deficiency operates to affect alimentary functions, we believe our records have established 1, that gastric atony is part of the syndrome characteristic of an advanced stage of vitamin B deficiency: 2, that successful vitamin therapy applied to such a case is associated with a rapid improvement in tone of the stomach musculature; 3, that the continued feeding of liberal amounts of vitamin B—enough to satisfy completely the body's requirement—to an organism subsisting on a diet adequate in other respects aids in maintaining a satisfactory gastric tonicity. We have therefore confirmed on dogs what the Japanese clinicians Ohomori, Ohhashi, Nakanishi, Hara and Ota (1922) noticed in human beriberi patients. We quote from their paper: "Movements of stomach. Examination of Y showed marked retardation and stasis of ten or more hours. There were several such cases."

The conditions which have been demonstrated thus far to produce or to favor the production of gastric atony are high fever, fever chills (Carlson, 1916) parathyroid tetany (Carlson, 1916), acute gastritis (Luckhardt and Hamburger, 1916), pneumonia, distemper and general peritonitis (Carlson, 1916). To this list must now be added prolonged subsistence on a diet low in vitamin B content. A dietary factor, therefore, may play an etiological rôle. In man this occurs particularly in cases of beriberi and perhaps, we venture to suggest, in other cases in which individuals, through force of circumstances or from faddist inclinations, have limited themselves in their choice of foods. Eddy and Roper (1917) have demonstrated that vitamin B therapy may prove advantageous for marasmic infants. In the light of our experiments it is possible that those babies who responded to Eddy and Roper's vitamin therapy may have been suffering from diminished gastric motility induced in part by faulty diet. It is quite possible also that certain types of convalescents owe their anorexia to too prolonged

subsistence on a diet low in anti-neuritic vitamin. This is a possibility which we feel deserves some attention by clinicians. It is certainly suggestive that a *dietary* factor has been demonstrated to be related to gastric atony.

The experiences gained in this study have caused us to realize even more fully than before the fact that the nutritive condition of animals used for experiments in physiology must not be overlooked; that the feeding of table scraps supplemented at rare intervals by better nutrients will very often fail to secure the ideal state of nutrition instead of gaining it. We strongly suspect that quite a few of the failures of different physiological laboratories to confirm one another's findings have been due to the experiments having been performed on animals that were not in what might be regarded as comparable states of nutrition.

SUMMARY

Four dogs having permanent gastric fistulae were fed on artificial diets adequate except with respect to vitamin B. From time to time the motility of the empty stomach was recorded by means of an inflated rubber balloon placed in the stomach and connected with a chloroform manometer. When the experimental animal lost the desire to eat, due to lack of vitamin B, several daily administrations of beef extract were made as a control experiment. This material does not contain the anti-neuritic vitamin and, when given in the amounts used in these experiments, promotes the flow of gastric juice in normal animals. When beef extract had been shown to have no effect on the anorexia, a vitamin preparation was given with a resultant recovery of appetite.

In mild cases of vitamin deficiency associated with anorexia there is no remarkable change in the character of the hunger contractions. The rhythmic tonus is frequently absent and the number of contractions in a series is somewhat less. In the severe cases of this deficiency in which anorexia is associated with nervous and muscular symptoms there is gastric atony. Successful vitamin therapy applied to such cases is associated with a rapid improvement in tone of the stomach musculature. Furthermore, the continued feeding of liberal quantities of vitamin B to an organism subsisting on a diet adequate in other respects, aids in maintaining a satisfactory gastric tonicity.

It is difficult, when dealing with such a parallelism as has been described, to decide whether or not vitamin B maintains the desire to eat simply by aiding in the preservation of the normal gastric tone. The marked systemic manifestations characteristic of advanced cases of vitamin B deficiency suggest that the loss of the desire to eat in such cases is due as much to a generalized systemic disturbance as it is to an abnormal condition localized in the alimentary canal.

BIBLIOGRAPHY

BEZNAK, A. v. 1923. Biochem. Zeitschr., cxli, 1.

CARLSON, A. J. 1916. The control of hunger in health and disease. Chicago.

COWGILL, G. R. 1921. This Journal, lvii, 420.

1922. Proc. Soc. Exper. Biol. and Med., xix, 282.

1923. Journ. Biol. Chem., lvi, 725.

COWGILL, G. R., H. J. DEUEL, JR. AND A. H. SMITH. 1925. This Journal, lxxiii, 106.

COWGILL, G. R., A. H. SMITH AND H. H. BEARD. 1925. Proc. Amer. Soc. Biol. Chem., Journ. Biol. Chem., lxiii, xxiii.

CRAMER, W., A. H. DREW AND C. MOTTRAM. 1921. Lancet, i, 963.

CRAMER, W., A. H. DREW AND C. MOTTRAM. 1922. Lancet, ii, 1902.

CRAMER, W. 1923. Lancet, i, 1047.

DAMON, S. R. 1922. Journ. Amer. Med. Assoc., lxxix, 128.

EDDY, W. H. AND J. C. ROPER. 1917. Amer. Journ. Dis. Child., xiv, 189.

FARMER, C. J. AND H. E. REDENBAUGH. 1925. This Journal, lxxv, 45.

GROSS, L. 1924. Journ. Path. and Bact., xxvii, 27.

IVY, I. C. AND G. B. MCILVAIN. 1923. This Journal, lxviii, 124.

KARR, W. G. 1920. Journ. Biol. Chem., xliv, 255.

LUCKHARDT, A. B. AND W. W. HAMBURGER. 1916. Journ. Amer. Med. Assoc., lxvi, 1831.

MCCARRISON, R. 1921. Studies in deficiency diseases. London.

OHOMORI, K., Y. OHHASHI, H. NAKANISHI, M. HARA AND T. OTA. 1922. Japan Med. World, ii, 128.

PAVLOV, J. P. 1902a. Ergebn. d. Physiol., i, 246.

1902b. The work of the digestive glands. Transl. by W. Thompson, London.

VEDDER, E. B. 1913. Beriberi. Wm. Wood & Co., New York.

EFFECT OF PHYSICAL EXERCISE UPON THE COMPLEX REACTION TIME AND AUDITORY ACUITY

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For an introduction to the subject of this paper one cannot do better than refer the reader to the very excellent and comprehensive summary of Spaeth (1919). In Spaeth's article will be found, in the section on "psycho-physiological methods," a discussion of the work of Kent (1915, 1916). The point of departure of the investigations to be described below is this work of Kent in which the latter presents a study of the effects of fatigue in laboratory workers and in industrial operatives. Among the methods employed by Kent two alone concern us. They deal with the complex reaction time and the acuity of hearing.

COMPLEX REACTION TIME. *Historical.* Kent tested three varieties of reaction time: 1, the simple reaction time (response to a stimulus); 2, discrimination time (response to one of two possible stimuli); 3, the complex reaction time, in which one of six colored keys is pressed in response to the exhibition by the operator of one of the same six colors. One or more of these methods was employed in studying the effects of the day's work on colliers, men from the chemical works and letter press printers. Among the last mentioned were a number of women.

On comparing the results obtained in the morning with those obtained in the afternoon, one finds that the latter are usually longer but that to this rule there is a considerable number of exceptions especially during the first part of the week and among women. The curves which in a general way show an increase in time toward the end of the week or a decrease in the morning to evening ratio are subject to great variation; sometimes the weekly curve constructed from the evening results shows a more even

[1] This article is based on the unpublished thesis of students majoring in the department of Physical Education and working in the department of Physiology under the direction of Prof. Percy M. Dawson, the compiler of this article. The theses referred to are: Fellows and Williams, A. B., 1919, The effect of a day's work of a physical education student upon the complex reaction time; Sammons, A.B., 1920, The effect of physical fatigue upon auditory acuity; Zimmerman, S.B., 1921, The effect of fatigue upon auditory acuity; Goldsmith and Luenzman, S.B., 1925, The effect of muscular fatigue upon auditory acuity.

decrease in efficiency than a curve similarly constructed from the morning observations. These methods were also used in the study of the effect of over-time in increasing the degree of fatigue. This demonstration is, however, much marred by irregularities though in the daily and weekly increases in reaction time the figures given are more striking than those referred to in the preceding paragraph. But the conclusions drawn by Kent from the results, Spaeth claims are unjustified since they are not borne out by a careful inspection of Kent's tables and charts, and it is certain that the many discrepancies render Kent's conclusions somewhat unsatisfactory.

Experimental. In view of the importance of this subject and the uncertainty in respect to it, Fellows and Williams performed a series of experiments in which the complex reaction time was determined. The results of these workers have lain hidden shyly awaiting the opportunity of appearing as a rider on the positive results of more fortunate though not more painstaking experimenters. The present seems a suitable occasion for presenting their work briefly.

The subjects of the experiments of Fellows and Williams, nine in number, were women students majoring in the department of Physical Education. Each was examined between 7:30 and 8:30 a.m. and again between 9:30 and 10:30 p.m. The program followed by each throughout the day was carefully recorded. Sometimes the program was exceptionally active involving a hockey match, a basket ball match or a swimming meet, at other times it involved only the routine intellectual work. In eight of the nine subjects it was found that the complex reaction time was less in the evening than in the morning. The morning times range from 0.555 to 0.789 second, average 0.662; the evening 0.529 to 0.731, average 0.616 second; while the differences between the morning and evening speeds of response were from 0.022 to 0.122, average 0.616 second, in the eight subjects. In the remaining subject there was an evening increase amounting to 0.105 second.

The subject showing an increase in complex reaction time was of a distinctly different type from the rest. Her scholastic standings were lower and in the gymnasium her powers of coördination were inferior. It would seem therefore that the quickening of the complex reacting time at night is to be regarded as the more normal phenomenon.

The obvious, practical conclusion drawn from these results was that the complex reaction time in the case of women students of the department of physical education is worthless as an index of the degree of fatigue produced by the activity of a single, even though sometimes rather strenuous, day, for the test, as already stated, showed for the most part an improvement in the reaction time at the end of such a day.

AUDITORY ACUITY: *Historical.* A second method employed by Kent is

that of auditory acuity, the test with the ticking watch. Using this method Kent examined 6 colliers, and 5 men from the chemical works. In these persons the auditory acuity was found to be least in the evening and at the end of the week. Tests made upon 6 letter press workers, 3 men and 3 women, showed great individual variation but otherwise the results resembled those obtained with the other workers. Kent also employed this method successfully in the study of the effects of overtime on 3 women doing machine work in an engineering factory and in a comparison of the effects of night and day work in five male makers of surgical lint. (Unfortunately the chart which illustrates the latter is a composite one involving not only auditory acuity but also visual acuity and reaction time.)

Experimental. It seemed to us that by far the most promising of the methods described by Kent was this of auditory acuity, and it is the primary object of the present article to present results obtained by this method in several series of experiments.

A. Diurnal and weekly variations (Sammons). The watch (always the same watch) was suspended against a small, vertical, padded surface which was slid by the observer back and forth along a wooden track marked in decimeters.[2] The subjects, 29 in number, alert and sympathetic, were for the most part women students majoring in the department of Physical Education. They reported in the morning between 7:30 and 9 o'clock, made a statement as to how they had spent the preceding 24 hours, and were then tested. For this purpose the subject was seated at the end of the track (already referred to) with her right ear turned toward the watch, which hung at about the level of the ear and which could not be seen by the subject.

Previously four distances had been selected, 120, 140, 160 and 180 cm., at which the watch could be heard by all the subjects when unfatigued, and now the observer moved the watch from one of these distances to another in an irregular manner so that the subject could form no idea (except through hearing) of her distance from the watch. The watch was left for 15 seconds in each position and the subject responded "yes" or "no" according to whether she heard the ticking or not. The number of trials was 40, ten at each position. From time to time the answers were checked by covering the watch with the hand so that it could not be heard by the subject; such a manoeuver was termed giving a "blank." The entire test occupied 12 to 15 minutes. Between 5:20 and 9:00 p.m.

[2] The reader may wonder why any one should employ so crude an apparatus as an ordinary watch moved back and forth in an ordinary room, seeing that the literature of otology, psychology and the special senses is full of descriptions of ingenious, elaborate and accurate methods of studying auditory acuity. (See, for example, Swan, 1923.) By way of explanation it should be said the principal motive underlying our experiments was the need of a criterion, a test, of fatigue which could be applied with the simplest and more readily available apparatus.

the tests were repeated. Finally the percentage of correct answers for each distance of the watch was determined. The results of these tests are presented in the accompanying table of averages (table 1) while a more detailed picture can be found in the second table (table 2) where the observations are divided into five groups in respect to the amount of decrease in acuity resulting from a day's activities.

The difference in favor of the morning hour shown in table 1 occurs in spite of the fact that the evenings are quieter than the mornings so that one would have expected an apparent increase of acuity at this time.

TABLE 1

Showing that in the evening the subjects fail more often to hear the watch especially at the greater distances

	DECIMETERS			
	12	14	16	18
Percentage of correct, affirmative answers:				
Morning	88	86	73	62
Evening	76	61	52	37

TABLE 2

CHANGE IN ACUITY OBSERVED IN THE EVENING	NUMBER OF SUBJECTS	FEELING OF SUBJECTS	DAY OF WEEK						
			S	M	T	W	T	F	S
1. Greatest loss	11	"Very tired"				1	4	4	2
2. Medium loss	4	"Tired" and "not very tired"		1	1	1			1
3. Slight loss	9	One "quite tired"							
		Two "tired"	1	3	1		2	1	1
		Four "not tired"							
4. No loss	2	Two fresher than in morning		2					
5. Gain in acuity	2			1				1	

From the second table it can be learned that of the 29 subjects, 25 showed a decrease in acuity, 2 no change and 2 an increase in acuity in the evening. Of the two subjects who heard better at night one stated that she did not feel awake during the morning test. The morning test of the second subject was not wholely satisfactory for she invariably answered "yes" to any position of the watch up to 24 decimeters—including blanks. These two cases are nevertheless included among the averages in table 1. A further perusal of the table shows that as a rule the acuity varies inversely as the subjective sensation of fatigue. There are some exceptions

toward the middle of the scale of acuities but on the whole the correspondence is very good.

In addition to the facts disclosed in table 2 there is, as further evidence of fatigue, the observation that the subjects hesitated more over their answers in the evening than they did in the morning and also made more errors when "blanks" were presented. Thus of 57 blanks presented in the morning 33 per cent were "heard" while of the 47 blanks presented in the afternoon 45 per cent were "heard," suggesting that the self deception may be one of the characteristics of fatigue.

No attempt was made to follow minutely the daily curve so that the observation of Bachrach (1916) that a maximum of acuity occurs at 6 p.m. has not been tested.

Although we made no special effort to determine the occurrence of weekly variations, still it may be seen (table 2) that the eleven observations which show the greatest diurnal variation were made during the latter part of the week.

That the test itself produced a certain degree of fatigue of auditory acuity seems to follow from the following figures. Of the 255 negative answers given in the morning 42.3 per cent were in the first half of the test and 57.6 per cent in the second; the 487 negative answers given in the evening 41 per cent occurred in the first half of the test and 58.1 per cent in the second half.

B. Effect of physical exercise. First series (Zimmerman). The method employed in these experiments differed from that employed by Sammons only in the manipulation of the watch. Here a point was selected at which the ticking of the watch could always be heard with ease, and the watch was then moved away a decimeter at a time until it was no longer heard. As soon as the subject gave a negative response the watch was moved back two decimeters. This kept the subject always slowly pushing back the watch as it were while the latter tended to come back more rapidly whenever the subject made an error. Hence random answers were excluded as these would have soon resulted in an irregular but inevitable and rapid movement of the watch in the direction of the subject. The forms of physical exercise, the effects of which were observed, were field hockey, riding on the stationary bicycle (half-hour or one hour) and alternately riding the latter and turning it by hand the break weight having been decreased.

Answers were sought to the following questions: 1, does auditory acuity decrease after the exercises in question? And 2, does the ear fatigue more rapidly during the test which is preceded by exercise?

The results justified answering the first question in the affirmative with the proviso that the exercise be severe enough. One subject (D) for example after half an hour's riding showed an increased acuity, (2 experi-

ments); when the time was raised to one hour his acuity was increased upon one occasion and diminished on another; when he alternately rode and turned the pedal by hand (a very severe effort) there was a pronounced decrease in acuity.

A second subject (Z) found an increase in acuity after field hockey (2 experiments), a variable result after half an hour on the bicycle (increase twice and decrease once) and a decrease after an hour's ride on the bicycle.

Of the four remaining subjects each of whom rode for half an hour the two who were unaccustomed to exercise and the one who became sick and dizzy from too great haste, showed a decrease in acuity after riding. The remaining, more athletic subject, showed an increase.

Turning now to the second question, whether exercise causes the acuity to fatigue more rapidly during the test, all that can be said is that in the 16 experiments which comprise this series there is no evidence at all of such a relation.

Second series (Goldsmith and Luenzman). The procedure in this series of experiments was similar to that employed by Zimmerman save for such details as follow. The tests were made every evening at the same hour when a reasonable silence prevailed. On each occasion the subject was given forty trials not counting "blanks." Upon each of the three subjects daily observations were made for from five and a half to seven weeks.

A distinction was at first made between the maximum acuity found during a test and the average acuity during this test, but it was soon observed that the two series of phenomena varied together and consequently the distinction ceased to have significance.

The results in this series extended and wholely confirmed those of the first series. In subject D a ride of one-hour on the cyclostat usually caused an increase in acuity while a walk of 30 miles or more decreased it. On one occasion only this subject showed a decrease in acuity after a half hour's ride. At the time he was unaware of anything abnormal in his condition but next morning he found that he had developed a severe cold. In subject L a 5-mile walk increased while 10 miles decreased the auditory acuity as did also a 5-mile walk plus one hour of gymnasium work. In subject G a 5-mile walk plus one hour of gymnastics raised the acuity but when a second hour of gymnastics was added the acuity fell.

The *cumulative effect* of exercise was also observed in this series. On one occasion subject G showed after exercise an increase of acuity but on the repetition of the exercise on the day following the acuity was decreased.

It was also observed incidentally that severe *mental activity* (e.g., preparing for or taking an examination) caused a decrease in auditory acuity.

C. Effect of practice (Goldsmith and Luenzman). The effect of practice

was very conspicuous. At first the watch was suspended in a small card board box which served to deaden the sound. Later a pad of fibrous material 2 cm. thick, intended for deadening the sound in buildings, was placed over the box. Moreover the chair of the subject which at the beginning of the series had been placed one meter from the apparatus had to be removed many meters as the hearing became more acute. Since, however, no precautions were adopted to prevent reflections of the sound from the walls and ceiling, the observations have only a qualitative value. One can say with assurance that the acuity is markedly increased but one cannot say how much it is increased.

CONCLUSIONS

From the experiments outlined above we feel justified in drawing the following conclusions.

1. The day's work of the women students of physical education results in an improvement (decrease) in the complex reaction time even when the schedule includes a basket ball match, a field hockey match or a swimming match. In nine subjects there was but a single exception.
2. The auditory acuity may be greatly improved by practice.
3. The day's work of a university student usually results in a decrease in auditory acuity which is more prone to be low towards the end of the week.
4. Light physical exercise results in an improvement in auditory acuity.[3]
5. Severe physical exercise results in a decrease in auditory acuity which is more marked the more severe the exercise.[3]
6. Exercise which is not severe on the first day may prove to be so when repeated on the following day.
7. The test itself produces some degree of auditory fatigue but no evidence was obtained to show that this decrease in auditory acuity comes on more rapidly in fatigued subjects.
8. At times the test of auditory acuity is more reliable than the subject's own sensations and indicates a physical depression of which the subject is not at the time aware.
9. As a practical means of detecting fatigue the test of auditory acuity is not wholly satisfactory because it requires more quiet than can usually be obtained.

BIBLIOGRAPHY

BACHRACH: Zeitschr. f. Psychol., 1916, xlix, 99.
BROWN: This Journal, 1925, lxxii, 241.
KENT: Interim report on an investigation of industrial fatigue by physiological methods, to British Parliamant, 1915, Cd. 8056; also Second interim report etc., 1916, Cd. 8335.
SPAETH: Journ. Indust. Hygiene, 1919, i, 23.
SWAN: Proc. Amer. Acad. Arts and Sciences, 1923, lviii, 425.

[3] It is a matter of interest in this connection to note that the knee jerk is subject to similar variations under the same conditions. (Brown, 1925.)

THE EFFECTS OF ASPHYXIA AND ISLETECTOMY ON THE BLOOD SUGAR OF MYOXOCEPHALUS AND AMEIURUS

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PART I—Observations on myoxocephalus.[1] That the reducing power of the blood of fishes immediately after catching varies both among individuals of the same species, and among those of different species, has been shown by several workers (Scott, 1921), (McCormick and Macleod, 1925). Although all of the factors responsible for these variations are as yet unknown, an important one has been shown by McCormick and Macleod (1925) to be disturbance in the respiratory function due to partial asphyxiation, as occurs in taking the fish out of water. In observations on Myoxocephalus they found that such exposure caused the blood sugar to rise in about thirty to forty minutes, the maximum degree of hyperglycemia being reached in about one hundred minutes. After replacement of the fish in sea water the blood sugar usually returned to normal within two days, but might not do so for four days. No parallelism could be detected between the degree of hyperglycemia induced by asphyxia and the amount of glycogen in the liver. These workers also investigated the behavior of the blood sugar in Myoxocephalus following the removal of the principal islets, an operation which can be readily carried out in the species without injury to the pancreas proper (zymogenous tissue). They found that a much higher degree of hyperglycemia supervened following this operation than in control fish exposed to air for the same time, or in others in which a mock operation was performed by exposing the viscera.

As the number of operated controls was somewhat limited, it was considered advisable to repeat the observations, since the results obtained furnish the most direct proof in support of the islet hypothesis for the source of insulin. The present paper records these results, and also others bearing on the effects of asphyxia and other conditions which influence the blood sugar.

[1] Part I represents work done as research fellow of the University of Toronto, and Part II as a student of the Honorary Advisory Council for Industrial and Scientific Research of Canada.

METHODS. The normal blood sugar value was taken as that obtained by the Shaffer-Hartmann method on blood drawn from the bulbus arteriosus, with needle and syringe, within one minute of the time of catching the fish. The samples of blood were collected and the proteins precipitated with sufficient rapidity to eliminate the use of oxalate or other anticoagulant.

A submarine pen or crib for holding fish in the sea was made as follows: a frame 6 feet × 6 feet × 3 feet was built of 2 inch × 4 inch timber, and covered with wire netting. A partition of wire netting divided it into two equal compartments which were provided with separate hinged covers of the same material. The whole was readily raised or lowered from a float by means of a windlass, and kept on a ledge of rock approximately 6 feet below low tide level, except when fish were being taken out or put in.

Unfortunately, sculpin (Myoxocephalus) were not available within the immediate vicinity of the pen. However, they were put in tubs of frequently renewed sea water immediately on being caught and thus kept until placed in the pen. The total time from catching of fish to placing in pen never exceeded one hour.

RESULTS. *Normal values.* The following normal blood sugar values were obtained on several occasions in fish immediately after catching with hand line.

JULY 8	JULY 9	JULY 10	AUGUST 31
0.090	0.012	0.028	0.040
0.085	0.016	0.026	0.033
0.083	0.008		0.035
0.088	0.016		0.035
0.088	0.028		0.035
0.098	0.020		0.028
0.083	0.024		0.030
	0.022		0.033
	0.020		0.033
	0.016		0.024
	0.010		0.026
	0.016		0.035
	0.020		0.035
			0.024

The exceptionally high values obtained on July 8, and which were amongst the first to be examined, cannot be accounted for. The estimations were carried out by precisely the same technique as on the other fish, and there was nothing unusual in the handling of the fish. That such unexpected high results should occasionally be met with, makes it necessary when investigating the behavior of blood sugar in fish, to use

large numbers of controls. Omitting the values of July 8, the average normal blood sugar is 0.029 per cent. The average of these results taken together with those of McCormick and Macleod is also 0.029 per cent. The average deviation from the mean of all available normal blood sugars for sculpin is 0.0087 mgm. per 100 cc.

Development of asphyxial hyperglycemia. The development of asphyxial hyperglycemia brought about by leaving fish in air after catching was next studied. The results of these observations are given in table 1.

TABLE 1

Development of asphyxial hyperglycemia

DATE	TIME AFTER CATCHING	BLOOD SUGAR
July	*minutes*	*per cent*
13	10	0.018
14	13	0.024
14	16	0.037
13	20	0.042
14	21	0.024
13	30	0.030
14	30	0.048
14	36	0.067
13	40	0.059
14	40	0.088
14	40	0.058
14	45	0.101
14	45	0.109
13	60	0.084
13	60	0.163
13	75	0.084
13	90	0.133
14	100	0.150
13	100	0.210
15	105	0.040
14	110	Very high
14	115	0.129
14	125	Trace
15	130	0.155
14	130	0.182
15	170	0.099
14	190	0.161

There is very little, if any, rise in blood sugar until twenty or thirty minutes after catching, which is a fact of importance in considering the cause of hyperglycemia due to other conditions. In about thirty minutes the blood sugar begins to rise and it continues to do so fairly steadily during the next thirty minutes, reaching to about 120 mgm. per cent in one hour. Subsequently it rose considerably higher (to about 200 mgm.

per cent) in some fish, and declined somewhat in others, so that the results obtained in different fish became very irregular after prolonged exposure.

Whether or not the blood sugar of a fish removed from water for one minute or so and then returned to the sea, would subsequently rise, was not directly determined, but from the results described on page 415 we find that if any rise did occur it has disappeared in three hours.

Recovery from hyperglycemia of catching. Having confirmed the fact that exposure to air causes a very appreciable rise in blood sugar it was necessary to find for how long fish must remain in a submarine pen after catching and transportation in tubs to the Biological Station, before their blood sugar would return to normal. From time to time after being placed in the pen fish were removed and sugar estimations made with results as follows:

Time in pen after being caught and placed there

1 HOUR	1 DAY	2 DAYS	3 DAYS	5 DAYS
per cent	*per cent*	*per cent*	*per cent*	*per cent*
0.062	0.024	0.020	0.026	0.024
0.062	0.028	0.033	0.037	0.037
	0.016			
	0.053			
	0.058			

With the exception of one fish, examined on the first day after being caught and placed in the pen, the blood sugars were all back to normal within one day. This is not in entire agreement with the work of McCormick and Macleod, who frequently found this hyperglycemia to persist for four or five days.

Recovery from asphyxial hyperglycemia. Before undertaking isletectomies it was desirable to know to what extent exposure to air for a period of time necessary for the operation might itself have on the blood sugar levels. For this experiment fish that had been in the pen for several days and in which, therefore, the blood sugar was normal, were wrapped in wet towels and exposed to air for twenty minutes and then returned to the pen. Several fish were removed on each of the six following days and sugar determinations made.

1 DAY	2 DAYS	3 DAYS	4 DAYS	5 DAYS	6 DAYS
0.040	0.077	0.048	0.024	0.056	0.024
0.032	0.086	0.056	0.028	0.056	0.024
0.048	0.028	0.028	0.020	0.024	0.024
0.044			0.018		

Since some food was found in the digestive tracts of the great majority of these fish it is highly unlikely that absence of glycogen is responsible for the usually observed normal blood sugars. It will be seen that generally the hyperglycemic effect of twenty minutes' asphyxiation has passed off in four days, but occasionally may persist a little longer. This confirms the work of McCormick and Macleod.

TABLE 2
Isletectomies

DATE	TIME SINCE OPERATION	BLOOD SUGAR
August	*days*	*per cent*
28	1	0.090
29	2	0.125
21	2	0.306
22	3	0.148
1	3	0.070
1	3	0.076
3	5	0.087
3	5	0.117
29	5	0.610
20	5	0.252
24	5	0.202
4	6	0.079
4	6	0.101
12	6	0.170
26	6	0.440
6	8	0.500
6	8	0.065
20	8	0.312
27	8	0.740
26	9	0.103
13	12	0.180
13	12	0.185
14	12	0.200
12	14	0.190
15	14	0.162
29	17	0.030*
20	21	0.109

* Small islet remaining in *post-mortem* examination.

Isletectomies. In the sculpin the islets of Langerhans are sufficiently isolated from the pancreas to be easily excised leaving the pancreatic tissue proper intact. The largest islet is bound to the spleen by a thin sheath of mesentery and can readily be discerned against the deep red background of the latter organ. A second large islet can usually be found in the mesentery in close proximity to the duodenum. Besides these main islets there are frequently smaller ones, scattered or in clusters, suspended in the mesentery within the duodenal loop.

The fish were wrapped in wet towels, leaving the abdomen exposed, and bound to a small board with cords, one just anterior to the pectoral fins and the other posterior to the anus. Tied in this manner the fish could be held quite firmly so that the operation was easily performed by one person. Starting from a point about 1 cm. from the right pectoral fin, the abdomen was opened carefully with a clean cut extending caudad for 5 or 6 cm. Very little bleeding is encountered if care be taken in making the incision. By retracting the abdominal walls the mesenteric islets can readily be brought into view. The larger ones were ligated with fine thread and then excised, and the smaller ones picked off with a fine forceps. With further retraction the splenic islet was exposed and

TABLE 3

Controls

DATE	TIME SINCE OPERATION	BLOOD SUGAR
August	*days*	*per cent*
22	2	0.062
27	2	0.062
27	3	0.062
28	3	0.050
28	3	0.050
21	3	0.077
22	4	0.033
21	5	0.057
25	5	0.037
24	6	0.040
25	6	0.048
25	6	0.020
31	6	0 040
24	7	0.012
26	7	0.079

after careful forcep dissection of its mesenteric sheath it was ligated and excised. The wound in the abdominal wall was closed with two layers of discontinuous sutures, the first including peritoneum and muscle, and the outer one the skin. The operation was performed on the float beside the raised pen so that the period the fish remained out of the sea could be reduced to a minimum and thus cut down the effect of asphyxiation. The complete operation required approximately fifteen minutes.

Other fish were used as controls, the operation being performed in exactly the same manner with the exception of the actual removal of the islets.

Fish were removed from the pen at varying intervals following the operation and sugar determined. Table 2 gives the results of isletectomised fish and table 3 of controls.

It is quite evident from a comparison of these two tables that isletectomy has a very pronounced effect on the blood sugar level of Myoxocephalus, the average of isletectomised fish being 0.209 per cent against that of 0.049 per cent for the operated controls.

The effect of muscular effort. It has been observed by Macleod and Noble that the normal blood sugar of sluggish sea-bottom fish is relatively low (Myoxocephalus 0.035 per cent) compared with that of active fish like brook trout (0.100 per cent). It was thought that muscular effort might be a factor responsible for this difference and also for the variations sometimes found in the case of fish of the same species caught under similar conditions. An attempt was made to induce muscular movement by attaching corks by short wires to the dorsal part of the operculum. At any time the crib was brought to the surface the fish were in active motion, but whether or not this condition obtained when resting on the sea bottom (in which case the fish would tend to be floated up against the wire netting of the cover), cannot be affirmed. The following columns give the blood sugar values for fish under the respective periods of "exercise."

1 DAY	2 DAYS	3 DAYS	4 DAYS
per cent	*per cent*	*per cent*	*per cent*
0.035	0.005	0.024	0.024
0.059	0.005	0.033	0.024
	.060	0.020	0.016
	0.010	0.004	0.016

All fish became pale within a few hours, and remained so throughout their period of "exercise." None of the above fish were fed during the experiment, nor had they been given any food for some days previous. On this account the possibility arose of their being glycogen-free, and therefore, showing no rise in blood sugar. Consequently another experiment was carried out in which the majority had food in the digestive tract at the time of killing. The results of this group are shown below.

2 HOURS	5 HOURS	10 HOURS	1 DAY	2 DAYS	3 DAYS	4 DAYS	5 DAYS
per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0.037	0.030	0.033	0.024	0.020	0.022	0.016	0.020

From this it appears that whether or not the fish have been fed has little influence on the behavior of their blood sugar level under the conditions here inflicted, and, again, no rise was produced by this method of exercise.

PART II—OBSERVATIONS ON THE FRESH WATER CAT FISH (AMEIURUS NEBULOSUS). Ameiurus has proved to be a readily obtainable and easily

kept variety of fish for experimental purposes. A number have been maintained in first class condition for three months in a large tank of running water in the basement. The blood sugars of several fish immediately after removal from the tank were as follows: 0.016, 0.012, 0.016, 0.028, 0.033, 0.024, 0.030 per cent. It will be seen that the normal blood sugar of Ameiurus is much the same order of magnitude as that of Myoxocephalus.

Since it was found in Myoxocephalus that the degree of hyperglycemia caused by asphyxia is not proportional to the liver glycogen (McCormick and Macleod, 1925), the following observations were undertaken on

TABLE 4

Asphyxiated fish

DATE	FREE SUGAR	AFTER HYDROLYSIS	METHOD OF HYDROLYSIS	METHOD OF ASPHYXIATION
October				
21	0.114			
21	0.053	0.083	$\frac{N}{16}$ HCl ½ hour	Limited water 19°C.
21	0.098	0.110	$\frac{N}{16}$ HCl ½ hour	
22	0.065	0.120	$\frac{N}{16}$ HCl 1 hour	Air 90 minutes
22	0.062	0.110	$\frac{N}{16}$ HCl 1 hour	
22	0.117	0.150	$\frac{N}{16}$ HCl 1 hour	
26	0.016	0.060	$\frac{N}{20}$ HCl 1 hour	Limited water 30 minutes
26	0.060	0.120	$\frac{N}{20}$ HCl 1 hour	Limited water 60 minutes
26	0.050	0.100	$\frac{N}{20}$ HCl 1 hour	Limited water 135 minutes
26	0.101	0.100	$\frac{N}{20}$ HCl 1 hour	Limited water 180 minutes
26	0.058	0.088	$\frac{N}{20}$ HCl 1 hour	Limited water 360 minutes
27	0.056	0.110	$\frac{N}{20}$ HCl 1 hour	Air 30 minutes
27	0.070	0.110	$\frac{N}{20}$ HCl 1 hour	Air 60 minutes
27	0.090	0.140	$\frac{N}{20}$ HCl 1 hour	Air 90 minutes
27	0.072	0.110	$\frac{N}{20}$ HCl 1 hour	Air 120 minutes
27	0.079	0.110	$\frac{N}{20}$ HCl 1 hour	Air 210 minutes

Ameiurus with the object of finding out whether the increased sugar might be derived from some source other than the glycogen of the liver.

It has been found that the percentage of sugar in fish blood is greatly increased (doubled or trebled) by hydrolysis with acid. If protein-free blood filtrates are similarly treated the increase is very much less. This fact pointed to the possibility of some protein-sugar "compound" or polysaccharide present in the blood which might account for the high blood sugar of asphyxia. To test this hypothesis the amount of sugar after hydrolysis has been compared with that caused by asphyxia, and asphyxial blood itself has been hydrolysed to see if any further increase in sugar might occur. It has been found that the asphyxial values often closely approximate those following hydrolysis (on the same bloods),

TABLE 5

Treatment of blood of partially asphyxiated fish

DATE	DIRECT BLOOD SUGAR	POST HYDROLYSIS BLOOD SUGAR	PERIOD OF STANDING	CONDITIONS	REMARKS
	per cent	*per cent*			
October 23	0.060		Immediate	In atmosphere of nitrogen	Mixed blood of three fish
	0.060		5 hours		
	0.050	0.132	23 hours		
29	0.020		Immediate	In atmosphere of nitrogen	Blood of three fish plus chopped heart
	0.025		5 hours		
	0.020		24 hours		
	0.020		24 hours		
November 10	0.048		Immediate	Standing anaerobically under heavy oil	
	0.048		45 minutes		
	0.044		90 minutes		
	0.044		3 hours		
	0.048		5 hours		
November 11	0.040		Immediate	Under atmosphere of CO_2	Blood thickened, but a representative sample was obtained
	0.040		90 minutes		
November 19	0.040		Immediate	Under atmosphere of CO_2	
	0.040		5 hours later		
24	0.065		Immediate		
	0.065		90 minutes	Under atmosphere of CO_2	
	0.063		3 hours		
30	0.044		Immediate	Under atmosphere of CO_2	
	0.044		1 hour		
	0.040		2 hours		

TABLE 6

DATE	FISH NUMBER	NUMBER* OF DAILY INJECTIONS (5 U INSULIN EACH TIME) PREVIOUS TO BLEEDING	BLOOD SUGAR	GLYCOGEN
			per cent	*per cent*
January 5	1	1	0.048	
	2	1	0.034	
	3	1	0.067	
6	44	2	0.053	2.3
	5	2	0.066	2.0
	6	2	0.056	2.1
7	7	3	0.083	2.5
	8	3	0.056	2.3

* The eight fish were given 5 U insulin each day, two or three removed daily and asphyxiated in air for one hour before bleeding. The blood sugar did not rise to the values obtained in fish having no insulin as reported in the first part of this report.

but they never exceed them, and they are usually decidedly lower. Table 4 gives these results. Several attempts were made to discover whether the mechanism of the asphyxial rise in blood sugar was confined to the blood itself, or must have the coöperation of the tissues. This was done by exposing the fish to a degree of asphyxiation just sufficient to start the process, and then following changes in the per cent of sugar *in vitro* in an anaerobic environment. For this we used, in turn, atmospheres of nitrogen and carbon dioxide under a covering of heavy mineral oil. In none of these cases was it found possible to cause a rise in sugar content above that present at the time of bleeding. Table 5 shows the results of these experiments.

It was found in confirmation of McCormick and Macleod, that insulin somewhat retards the rise in blood sugar due to asphyxia. This is shown in table 6.

SUMMARY AND CONCLUSIONS

1. In Myoxocephalus the normal blood sugar immediately after catching the fish may vary from a trace to 37 mgm. per cent. It averages 0.030 per cent.

2. The exposure of fish to air causes a rise in blood sugar which appears after twenty to thirty minutes, and usually reaches about 0.120 per cent in about one hour.

3. The exposure to air for about 20 minutes which is the time necessary for the operation of isletectomy also causes hyperglycemia which may last for four or five days after replacing the fish in the sea.

4. Removal of the principal islets (isletectomy) is followed by hyperglycemia, which both in its magnitude and duration far exceeds that which can be attributed to exposure of the fish to air or to a mock operation similar to isletectomy, but without actual removal of the islets.

5. Attempts to produce hyperglycemia by inducing muscular effort were unsuccessful.

6. *In Ameiurus*, the normal blood sugar of fish kept in a tank is practically the same as in Myoxocephalus and it rises during exposure of the fish to air in the same manner.

7. When the blood is hydrolysed in Ameiurus the blood sugar increases markedly, and since this increase is relatively much less after asphyxia, it is considered possible that the extra blood sugar in asphyxia is due to break down of these compounds.

BIBLIOGRAPHY

McCormick, N. A. and J. J. R. Macleod. 1925. Proc. Roy. Soc. B, xcviii.
Scott, E. L. 1921. This Journal, lv. 349.

PARTIAL DESTRUCTION OF THE SINO-AURICULAR NODE IN DOGS' HEARTS BY EXCISION AND LIGATION

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This study was begun to determine the amount of sino-auricular node that might be destroyed in the dog's heart, and still have that structure retain its pace-making function.

HISTORY. Following the description of the Bundle of His in 1893, and the subsequent descriptions of the auriculo-ventricular node by Tawara (1906) and the sino-auricular node by Keith and Flack (1907), the relative importance of these structures in relation to the origin and conduction of the cardiac impulse became apparent. Gradually the conception evolved that the cardiac impulse was generated at the sino-auricular node, a bit of specialized tissue, representing the embryological remains of the sinus venosus, and located at the junction of the superior vena cava and the right auricle in the mammalian heart. The exact nature and method of spread of this impulse or excitation wave from the sino-auricular node to the auriculo-ventricular node is still uncertain. From the auriculo-venticular node, it is believed that the impulse is transmitted down through the branches of the Bundle of His to the Purkinje cells, thus reaching all parts of the endocardial surface and producing ventricular contraction.

To determine the unusual and remarkable nature of the nodal tissues, various methods, unfortunately artificial, have been employed among which may be mentioned the application of heat, cold, chemicals and cautery to the nodal tissues as well as complete and partial excision of the sino-auricular node.

The production of "nodal rhythm," i.e., a rhythm in which the impulse originates in the auriculo-ventricular node, was observed after excision of the sino-auricular node in mammalian hearts by Fredericq (1901), Langendorff and Lehmann (1906), Erlanger and Blackmann (1907), and Cohn, Kessel and Mason (1912).

Flack (1910) observed little or no change in rhythm after clamping and ligation of the sino-auricular node.

Wybauw (1910), and Lewis, Oppenheim and Oppenheim (1910) almost simultaneously attempted to locate the seat of impulse initiation in the

normal mammalian heart using the galvanometer or initial negativity method. Wybauw concluded that the impulse originated in the mid-portion, while the English workers concluded that it originated in the cephalic portion of the node. The latter has become the generally accepted theory.

Ganter and Zahn (1912) observed a marked reduction or disappearance of the As-Vs interval after destruction of the sino-auricular node, and suggested that the auriculo-ventricular node had taken over the function of impulse formation. Zahn (1913) further concluded that the length of the As-Vs interval after destruction of the sino-auricular node depended upon the portion of the auriculo-ventricular node which was originating the impulse.

Meek and Eyster (1914) produced nodal rhythm in mammalian hearts by the application of formalin, by crushing, cutting or clamping around the sino-auricular node, or by vagal stimulation. Continuing their studies, Eyster and Meek (1917) observed that in partial or complete removal of the sino-auricular node, the impulse in the nodal rhythm thus produced arose in the coronary sinus portion of the auriculo-ventricular node as determined by the method of initial negativity. The same authors (1922) ligated and excised the sino-auricular node confirming their previous studies, and noted that the impulse occasionally seemed to originate in the sino-auricular node despite thorough attempts at ablation of this nodal tissue. Ligation, per se, produced nodal, coronary sinus, or sino-auricular block rhythm with ultimate resumption of normal rhythm.

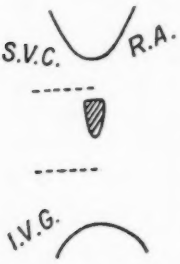

Fig. 1

This present study was begun to confirm or disprove the above findings, and to determine the exact amount of sino-auricular node tissue that it was possible to destroy, as accurately as it could be computed, and still have that structure retain its pace-making function.

Method. Under sterile precautions, 4 to 6 cm. of the third or fourth rib in each of 14 dogs were resected. Intermittent intratracheal air-ether insufflation was obtained by means of an Erlanger tank. The right pleural cavity and the pericardial sac were opened. A suture passing through the fat in the auricular-ventricular groove was utilized to rotate the heart on its long axis, thus swinging the postero-lateral aspect of the heart into view. Single over-lapping mattress ligatures of fine silk were laid in the area of the sino-auricular node and tied. The area thus tied off was excised with a knife or scissors. The wound was closed and the effect of the operation studied for a period of several months by means of the electrocardiograph. The excised tissue and the cardiac tissue about the site of excision were later studied histologically. Iron hematoxylin

and Van Gieson staining were used to identify the node. Sectioning was done at right angles to the long axis of the node, orientation being preserved by cutting through the superior vena cava and right auricle in such a way as to always reveal the exact junction of the latter.

DATA AND DISCUSSION. In the following presentation of data only the protocol on dog 1 together with a diagram of the sino-auricular node region have been included. A verbal summary and discussion of findings in each of the experiments simplifies the presentation.

In figure 1, the superior vena cava, *S.V.C.*, right auricle, *R.A.*, and inferior vena cava, *I.V.C.*, are labelled. The sino-auricular node is blocked in lightly. The upper and lower limits of the silk sutures are indicated by dotted lines.

Dog 1. As illustrated in figure 1, nodal tissue 2.55 mm. in length was found at autopsy. This bit of tissue was enclosed above and below with silk sutures. It was estimated at the time of operation that possibly the upper one-fourth of the node was excised. Histological examination of the excised tissue, however, revealed no nodal structure.

Electrocardiographic summary

RECORD FIGURE	CONDITION	TIME AFTER OPERATION	P-R INTERVAL	RATE
PR1	Normal		0.12	190
2	Normal		0.11	125
3	Normal		0.11	90
4	Normal		0.10	84
5	2 minutes' exercise		0.10	118
6	Atropin sulphate gr. $\frac{1}{60}$ intramuscularly		0.09	161
7	Exercise		0.12	120
8	Atropin		0.12	139
PO1	Normal	1 hr.	0.13	89
2	Normal	6 hr.	0.09	150
3	Normal	15 hr.	0.09	118
4	Normal	23 hr.	0.09	70
5	Normal	39 hr.	0.11	120
6	Normal	40 hr.	0.11	124
7	Normal	48 hr.	0.09	82
8	Normal	64 hr.	0.10	98
9	Normal	3 days		107
10	Normal	3½ days	0.09	138
11	Normal	20 days	0.11	88
12	Normal	24 days	0.11	91
13	Normal	31 days	0.10	114
14	Normal	40 days	0.10	112
15	Normal	57 days	0.13	85
16	After 2 minutes' exercise	57 days	0.11	97
17	Atropin	57 days	0.10	180

This animal was killed and autopsied on the 57th day.

In the tabulated electrocardiographic study record PR1 was a normal record. The animal was somewhat nervous at the time the record was taken, it being his first experience in the recording room. The following three records, PR2, PR3 and PR4 have an average p-r interval of 0.106 second. The atropin record was taken when the drug had produced a rate of 161, and the p-r interval had decreased to 0.09 second. The two minutes of exercise as well as the atropin produced some change in the p-r interval as well as in the rate. Neither appear to be significant, however. In this, as in all the animals, each administration of atropin consisted of grain $\frac{1}{50}$ atropin sulphate injected into the gluteal muscles. The exercise consisted of two minutes running on a leash, the record being taken as soon thereafter as possible.

Approximately 19 per cent of the node was found present at autopsy, although no nodal tissue was found in the excised portion. We are unable to explain satisfactorily the disappearance of the major portion of the node, unless it was due to necrosis following the puckering of the tissue ligated.

Dog 2. Only 1.2 mm. in length of nodal tissue was found at autopsy. This tissue was located 0.96 mm. below the superior cavo-auricular junction, and was entirely surrounded by sutures. The area excised contained nodal tissue. The post-operative p-r interval varied from 0.08 to 0.13 of a second which might be interpreted as abnormal, and suggests a nodal disturbance the exact nature of which cannot be stated. From our histological examinations we believe that the nodal tissue excised came from the lower part of the body and tail of the node.

Dog 3. Nodal tissue 3.54 mm. in length located at the head of the node was found at post mortem. Sutures were found passing directly through this tissue, and enclosing theoretically the tail, body and part of the head of the node. The electrocardiograms revealed the presence of nodal rhythm, and a gradual return to an essentially normal rhythm at the end of 12 days. It is possible that in this animal we have the phenomenon of replacement of the normal sino-auricular function as the pacemaker of the heart by that portion of the auriculo-ventricular node situated about the mouth of the coronary sinus as described by Eyster and Meek (1917).

Dog 4. Nodal tissue 1.19 mm. in length was found at post mortem. The upper limit of nodal tissue was 2.156 mm. from the superior cavo-auricular junction. The excised portion contained no nodal tissue. The electrocardiograms showed no change in the p-r interval after operation. Sutures were found above and below the nodal tissue that remained, and most of the node must have been tied off. In this instance, we feel that there has been no demonstrable effect following a complete ligation of the node.

Dog 5. Five and seven-tenths millimeters of nodal tissue remained at post mortem. Sutures were found passing directly through and enclosing most of this tissue. The excised portion revealed no nodal tissue. Nodal rhythm developed immediately after the operation. Unfortunately, the early death of the animal, from pneumothorax, prevented further tracings. We feel that inasmuch as the excised portion contained no demonstrable nodal tissue, the function was completely depressed by the sutures tied about the node.

Dog 6. Ten and seven-tenths millimeters of nodal tissue were found at post mortem. Only a few sutures were found in place close to the superior cavo-auricular junction. In view of the fact that the excised portion contained nodal tissue, and the sutures were found at the head of the node, we believe the portion excised to have been from the superior part or head of the node. It had also been estimated at the time of operation that the cephalic portion had been excised. The author believes that there were sutures below the point where they were found, which perhaps were lost in sectioning. A slight increase from 0.10 to 0.12 of a second in the p-r interval

was observed. This might be considered an indication of disturbed function in the node.

Dog 7. Four millimeters of nodal tissue were observed under histological examination. The upper suture passed through this nodal tissue, while the lower one passed, no doubt, below the caudal portion of the node. The excised portion contained nodal tissue, which we consider to have been from the body and tail of the node. The p-r interval is difficult to interpret. Eighteen hours after operation it was 0.07 of a second. Inasmuch as the pre-operative records show a p-r interval ranging from 0.12 to 0.08 of a second, the above figure cannot be considered very significant. However, as the p-r interval remained at a relatively low figure, it may be proper to consider this as another case of depressed functional activity of the node.

Dog 8. In this animal the upper margin of the node was found exactly at the junction of the superior vena cava and the right auricle, and extended downward for a distance of 5.1 mm. The upper suture passed through the body of the node; the lower suture passed below the tail of the node. The excised portion which was considered at the time of operation to come from the body of the node, contained nodal tissue. Five hours after operation the p-r interval had decreased to 0.06 of a second. We believe that a part of the body of the node had been excised, and that the decreased p-r interval suggests a depressed functioning node.

Dog 9. The histological study in this animal failed. The electrocardiographic records suggested a depressed but functioning sino-auricular node.

Dog 10. In this animal 3.3 mm. of nodal tissue were found at the site of the head of the node. The sutures passed above and well below the nodal tissue. The excised portion contained nodal tissue. No significant changes in the p-r interval occurred. It was roughly estimated that 24 per cent of the node had remained. No doubt, much less than 76 per cent of the node had been excised, but the displacement of nodal tissue incident to the suturing accounts for the above figures, which are considered inaccurate. We believe that this is a typical example illustrating that a part of the node may be excised without any loss in function apparent with our present means of detecting such a phenomenon.

Dog 11. Seventy-seven hundredths millimeter of nodal tissue were observed histologically, completely surrounded with sutures. The excised portion contained nodal tissue. It was estimated at the time of operation that the cephalic portion of the node had been excised. Histological examination corroborated this view, and suggested further that part of the body and tail were also excised. The p-r interval showed a definite decrease. We believe that there was a marked disturbance in the normal rhythm in this animal, and that the pacemaker while remaining in the node, had been displaced within that structure.

Dog 12. Eight and six-tenths millimeters of node remained at post mortem. The histological study of the excised portion failed. Sutures were found passing through the body of the node, and below its inferior limit. Partial heart block developed, with inverted "T" waves, and extra-ventricular systoles. It was concluded that here, too, the normal physiology of the origin of the cardiac impulse had been interfered with. Because more than 50 per cent of the node remained at post mortem, it was believed that the disturbance was essentially due to the suturing.

Dog 13. Seven and eight-tenths millimeters in length of nodal tissue located just below the superior cavo-auricular junction were found at post mortem. The sutures at their upper and lower limits passed through this tissue. The excised portion was found to contain nodal tissue. It is believed that part of the body of the node had been excised, which is borne out by the marked disturbance of rhythm as revealed in the electrocardiographic studies. This disturbance was evidently temporary and the heart soon recovered its normal rhythm and normal p-r interval.

Dog 14. Two and three-tenths millimeters of nodal tissue remained at the cephalic portion of the node. The sutures completely enclosed this bit of tissue The histological study of the excised portion failed, and we are, therefore, unable to state how much of the disturbance of rhythm could be attributed to the excision. Both excision and ligation, no doubt, contributed to the effects produced.

From the foregoing data, the following may be noted:

1. In each of these experiments, with two exceptions, the p-r interval was disturbed.

2. Nodal rhythm developed in three experiments.

3. The relation of the silk sutures to changes in rhythm is difficult to interpret because of accompanying excision in each instance. However, in those cases in which the excised portion contained no nodal tissue it was found that whereas complete ligation in one case produced no change, practically complete ligation in another case produced nodal rhythm.

4. Concerning the amount of nodal tissue left after ligation and excision in relation to changes in rhythm, we note that in three instances sino-auricular nodal tissue varying from 1.3 mm. to 5.7 mm. in length were insufficient to retain the pacemaking function. However, in five other experiments, nodal tissue varying from 1.19 to 5.11 mm. in length were sufficient to support sinus rhythm.

The histological and electrocardiographic results are tabulated in table 1, which is self explanatory.

Examination of our data on the effect of atropin and exercise upon the p-r interval and heart rate before and after operation, reveals the following:

1. That post-operatively, under atropinization, there were no instances of a decrease in the p-r interval, but rather that a slight increase was noted in four dogs.

2. That post-operatively under atropin there were three instances in which the heart rate never exceeded that reached under atropin prior to the operation.

3. That the normal heart rate was decreased in four animals and increased in three animals after operation.

4. That the figures for the exercise study are too few to warrant any statement.

Finally, we wish to point out that in one animal nodal rhythm developed post-operatively and continued for eight days when a permanent rhythm was established, the p-r interval ultimately being the same as that before operation. We interpret this as a case in point where true sinus rhythm returned illustrating the remarkable return of function of the sino-auricular node after cessation of that function during a period as protracted as eight days.

An exact estimate of the amount of destruction committed upon the sino-

TABLE 1

Summary of histological and electrocardiographic findings

Number	Histology in terms of millimeters						Electrocardiography					
	Length of nodal tissue	Distance below superior cavo-auriculo junction				Excised nodal tissue	Disturbed			Destroyed sino-auricular node		
		Nodal tissue		Sutures			Nodal rhythm	P-R interval	Heart rate	Post mortem after operation	Estimate at time of operation	Estimate after histological examination
		Superior	Inferior	Superior	Inferior							
												per cent
1	2.55	1.582	4.130	1.190	5.538	−		+	Normal	57 days	Upper 25 per cent	81
2	1.19	0.966	2.156	(above) 0.210	10.192	+		+	Normal	48 days	Upper 50 per cent	91
3	3.542	0.406	3.948	2.366	20.412		+	+	Decreased	60 days	All	74
4	1.19	2.156	3.346	0.406	17.080	+			Decreased	27 days	All	91
5	5.698	2.156	7.854	2.156	6.286	−	+	+	Normal	24 hours	Just below head	58
6	10.726	5.502	16.228	0.602	1.911	+		+	Normal	46 days	Head	22
7	3.92	0.994	4.914	2.758	16.494	+		+	Normal	49 days	All	72
8	5.11	At junction	5.110	3.552	7.266	+		+	Normal	32 days	Part of body	63
9								+	Normal	12 hours		
10	3.332	0.798	4.130	0.602	14.714	+			Increased	6 days	All	76
11	1.372	0.406	1.778	0.210	5.502	+		+	Normal	3 days	Head	90
12	8.624	0.406	9.030	3.452	11.186			+	Normal	6 days	Part of body	37
13	7.84	0.210	8.050	0.210	4.522	+		+	Decreased	7 days	Head	43
14	2.352	0.602	2.954	0.602	9.030		+	+	Normal	36 hours	Upper 50 per cent	83

auricular node was difficult and is considered unsatisfactory for the following reasons:

1. The exact size of the node in each dog previous to the operation was not known, nor do we know of any method whereby such data might be obtained.

2. While our data are quite accurate to within 14 micra in so far as the length of the node is concerned, both the width and thickness of the node are a matter of estimate.

3. The bit of excised tissue does not represent in each case the amount of destruction accomplished upon the node for the reason that the sutures undoubtedly cut off the blood supply of the tissue surrounding the excised portion, resulting in necrosis, and we have no method of computing the exact amount of such necrosis.

4. The large amount of suture material presents many difficulties in sectioning the post-mortem tissue.

5. The great amount of connective tissue proliferation following healing of the wound, and the resultant displacement of the remaining nodal tissue, make orientation difficult.

6. That no allowance has been made in computations for the shrinkage which the tissues must undergo in the process of histological preparation.

SUMMARY. In this series of fourteen experiments we have attempted to determine by histological methods and the electrocardiograph the exact amount of the sino-auricular node in dogs' hearts that could be ligated and surgically excised with a preservation of the normal pace-making function by the remaining portion of the node. These experiments were carried out under aseptic conditions using ether anesthesia, and allowing the animals to recover for a period varying from twelve hours to fifty-seven days.

The histological study consisted of the preparation and study of both the excised tissue, which in most instances verified our belief that we had excised a part of the node, and the post-mortem cardiac tissue which surrounded the excised portion. Van Gieson's method of staining with an iron hematoxylin nuclear stain was used.

The heart rate, p-r interval, and any cardiac disturbances were noted both before and after the operation. Atropinization of the vagus nerve was also carried out before and after the operations.

CONCLUSIONS

1. By ligation independent of excision, or by ligation and partial excision of the sino-auricular node in dogs' hearts, we are able to produce changes in the p-r interval, as well as a definite nodal rhythm, as interpreted in the electrocardiogram.

2. A considerable portion of the sino-auricular node in dogs' hearts may be excised, and the node continue its pace-making function.

3. There is a marked individual variance in the amount of node essential to its pace-making function.

4. The method herein used is unsatisfactory in attempting to determine the amount of the node that can be destroyed and have retention of its pace-making function.

5. The relatively unsatisfactory results obtained in this study have furnished the stimulus to seek another method. Destruction of the node by means of radium emanation will furnish the basis of a second paper to be published in the near future.

BIBLIOGRAPHY

COHN, A. E., L. KESSEL AND H. H. MASON. 1912. Heart, ii, 311.

ERLANGER, J. AND J. R. BLACKMANN. 1907. This Journal, xix, 125.

EYSTER, J. A. E. AND W. J. MEEK. 1917. This Journal, xlii, 611.
1922. This Journal, lxi, 117.

FLACK, N. 1910. Journ. Physiol., xli, 64.

FREDERICQ, L. 1901. Acad. r. d. Belg. Bull., I. des Sciences, 125.

GANTER, G. AND A. ZAHN. 1912. Zentralbl. f. Physiol., xxv, 762; Pflüger's Arch., cxiv, 335.

HIS, W. 1893. Abhandl. d. Sachs. Ges. d. Wiss. Math.-Physik.-Kl, xix, 1.

KEITH, A. AND M. FLACK. 1907. Journ. Anat. and Physiol., xli, 172.

LANGENDORFF, O. 1906. Pflüger's Arch., cxii, 522.

LEWIS, T., B. S. OPPENHEIMER AND A. OPPENHEIMER. 1910. Heart, ii, 147.

MEEK, W. J. AND J. A. E. EYSTER. 1914. Heart, v, 227.

TAWARA, S. 1906. Das Reizleitungssystem des Saügeteirherzen. Jena.

WYBAUW, R. 1910. Bull. d. l. Soc. Roy. d. Sc. d. Brux., no. 5; Arch. internat. d Physiol., x, 78.

ZAHN, A. 1913. Pflüger's Arch., cxli, 247.

ON THE RELATION OF THE AFTER-NYSTAGMUS TO ROTATION-NYSTAGMUS

I. Direct Effects of Rotations

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Although the investigations of the after-nystagmus are very numerous, there is but little work which concerns itself with the relation of the rotation-nystagmus to the after-nystagmus. It is necessary before any inferences from the studies of the after-nystagmus can be made, to know in what way the reactions during rotation affect those occurring upon the cessation of rotation.

In the present paper the rotation-nystagmus has been particularly studied and also the relation of the rotation-nystagmus to the after-nystagmus in regard to the effect of 1, duration of rotation; 2, repeated rotation; 3, cerebral injuries; 4, one-sided labyrinthine lesions, and 5, light.

In the experiments herein reported, an electrically-driven rotation table was used. It attained a uniform velocity during the second rotation. The table was provided with a brake, manipulated by the unwinding and winding of a cable about the propeller shaft, according to the method devised by Maxwell (1922). At the start of the rotation of the table, the cable unwound itself ten times and then wound about the propeller shaft ten times. The brake, being provided with a spring, was pulled away from the table at the beginning of the rotation and was automatically pulled against the table at the time calculated to bring about the stop. The motor was shunted out during the last rotation, in order that the stopping of the table should not be too sudden.

Ordinarily each animal was given ten complete revolutions to the right and ten to the left at a velocity of one revolution in two seconds. In some of the experiments twenty complete revolutions or even a greater number were employed. In the tables which are given and in subsequent discussions, one rotation signifies that the animal has been subjected to either ten or twenty complete revolutions both to the right and to the left.

The entire set-up to be described below was arranged upon the rotating table.

The animal was wrapped in cloth and placed in a close-fitting box, with

its head fastened in a Czermak head-holder. Movement of the body was prevented by filling the space between the animal and the box with towels. This obviated the necessity of tying the animal and yet prevented it from struggling or suffering any appreciable amount of discomfort.

The cornea was anesthetized with a few drops of two per cent cocaine solution, excess solution being wiped away, and the eyelids were propped apart by a speculum. An eye-cup of plaster-of-Paris, a modification of the eye-cup of Delabarre (1897–98), was placed on the anesthetized cornea and connected with a lever (see fig. 1), arranged to mark on the horizontally-placed kymograph. Time in seconds was indicated on the drum by a Jacquet chronometer and the moment of cessation of rotation was recorded by a signal magnet, connected with knock-over keys, which

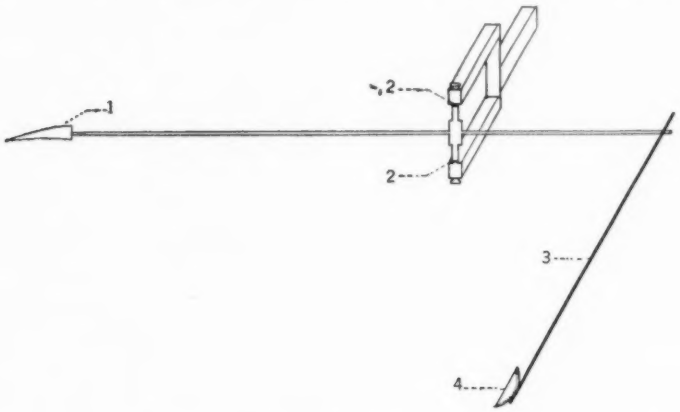

Fig. 1. Recording apparatus: *1*, writing point; *2*, pivots; *3*, flexible straw; *4*, eyecup.

were manipulated by a nail driven into the automatic brake. (See fig. 2.) It was found unnecessary to make the eye-cup with a radius of curvature the same as that of the eye-ball. For molds the bottoms of test-tubes were used. The same eye-cup could be used in numerous experiments, if care was taken to prevent it from becoming too moist.

The animals were of the New Zealand Red Variety and weighed between three and five pounds. None of these animals had been previously rotated. Animals with observable ear infections or showing irregularities in reactions as manifested by irregular eye-movements were discarded. In addition, animals which were restless or persisted in struggling were not used.

The rotating table with its various attachments and with the rabbit in position is shown in figure 3.

The above method of recording graphically the eye-movements excludes the personality of the observer and consequently increases the value of the results from a quantitative viewpoint.

A. Tracings of rotation-nystagmus and after-nystagmus. Examples of the tracings which are obtained by the above-described method are shown in figure 4.

Upon rotation to the left, the tracings clearly show that the direction of the compensatory phase of the rotation-nystagmus is opposite to that of the

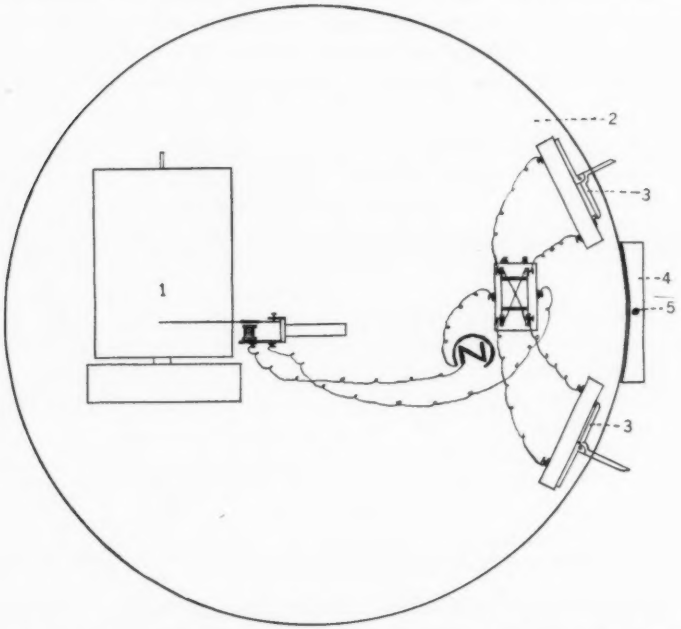

Fig. 2. Arrangement for recording cessation of rotation: *1*, kymograph; *2*, rotating table; *3*, knock-over key; *4*, brake; *5*, nail driven in brake to break circuit.

compensatory phase of the after-nystagmus; the same is true of the compensatory phases in the opposite direction of rotation, and therefore the direction of the compensatory phase of the rotation-nystagmus during the rotation to the right is the same as the direction of the compensatory phase of the after-nystagmus, following rotation to the left. Changing rate of the nystagmus is also shown by the spreading of the record of the nystagmic jerks as the rotation progresses. The typical spreading of the after-nystagmus is also clearly depicted.

In the subsequent tables, the duration of the labyrinthine excitation

usually is expressed in terms of number of eye-movements. The records make it possible to study both the number of movements and their duration, but in this work only the former is considered.

B. Comparison of first and second rotations. When the responses of the first rotation are compared with those of the second, the majority of the animals show less compensation during the second rotation than during the first. This is more striking in the animals which have been given twenty turns to each rotation (table 2), than in those which have been given only ten turns to each rotation (table 1). It also became evident that there are distinct variations in the responses following rotation to the right and to the left, a matter to be considered later on.

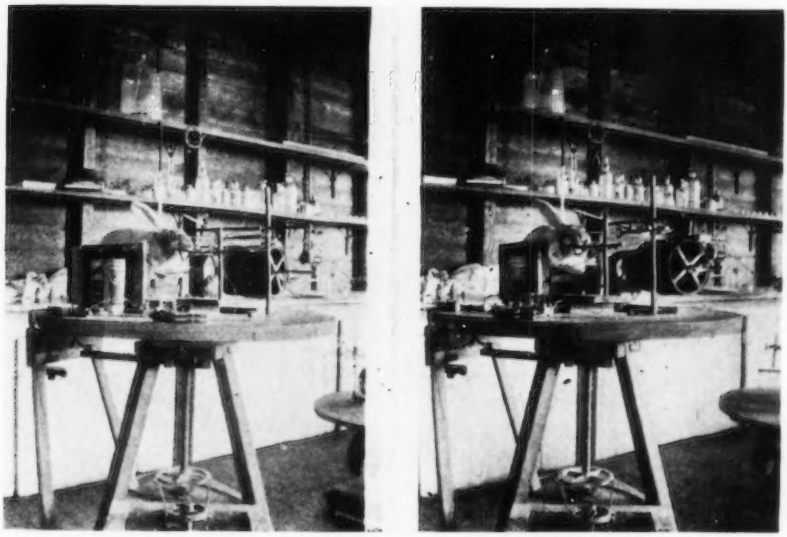

Fig. 3. Showing arrangement of animal and recording apparatus on the rotating table.

The fact that there is generally less compensation during the second than during the first rotation is interpreted as indicative of modification of the responses rather than as a deficiency of the method. To what extent the eye-cup interferes with the normal eye-movements is unknown, but since it is small and light and since the inertia of the lever is trifling, it does not seem probable that the eye-movements are altered to any appreciable extent. These observations show the readiness with which modifications of the response to labyrinthine excitation occur and the necessity for obtaining records of the first rotation of the animal, if modifications of the animal's responses are to be treated in a quantitative way.

The experiments of Hoshino (1922) are pertinent in connection with the above, for he found variations in the responses from day to day. He considered only the after-nystagmus, but it will be shown in another place that modification of the after-nystagmus occurs more readily than of the rotation-nystagmus. De Barenne and de Kleyn (1923) also com-

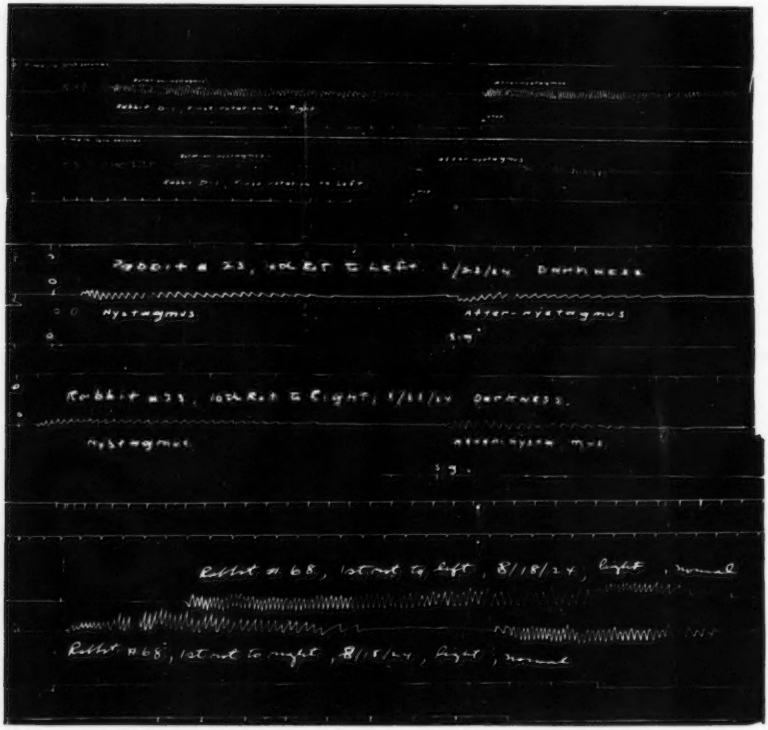

Fig. 4. Tracings of rotation-nystagmus and after-nystagmus. Rabbit D-1 was subjected to twenty turns to each rotation, whereas in rabbits 23 and 68, there were only ten turns to each rotation.

ment upon the variability of the after-nystagmus and the fact that the responses in the two directions of rotation are seldom the same.

C. Effect of repeated rotation upon the rotation-nystagmus and the after-nystagmus. The method of procedure was as follows: The animal was given ten complete turns to the right at a velocity of one turn in two seconds, and after a rest interval of from thirty to sixty seconds, ten turns to the left. This was repeated ten times and constituted one day of

practice rotation. Graphic records of the first and in some cases of the second rotation were taken and also records at the end of each day of practice rotation. The results, obtained from five rabbits, rotated under similar conditions, are plotted in figure 5. The percentage reductions are used as ordinates, and the practice days as abscissae. The amount of response was recorded at the end of each day of practice rotation.

The rotation-nystagmus rapidly declines during the first two days of practice and less rapidly on the following days. In none of the experiments has an obliteration of the rotation-nystagmus been observed. Rabbits 23, 24 and 26 were given more than 4000 rotations and still showed about 40 per cent of the original amount of the rotation-nystagmus. In some instances, after a variable amount of reduction had occurred, with further

TABLE 1

Comparison of first and second rotations. Ten turns to each rotation. Letters R and L denote turning to right and to left respectively. Average of 13 animals

NUMBER OF ROTATION	NYSTAGMUS		AFTER-NYSTAGMUS	
	R	L	R	L
1	55	62	37	30
2	52	58	33	28

TABLE 2

Same as table 1, except that the animals have been given twenty turns to each rotation. Average of 10 animals

NUMBER OF ROTATION	NYSTAGMUS		AFTER-NYSTAGMUS	
	R	L	R	L
1	124	121	66	35
2	116	111	64	27

rotation there was an increase in the response. In other words, instead of a definite stationary amount of reduction occurring, the response fluctuated between a low and a high level. This is shown in figure 5 by rabbit 84 (right) and rabbit 85 (right and left).

Figure 5 shows strikingly that the reduction in the after-nystagmus during the second to the fourth days of practice rotation is so great as practically to abolish it. When total cessation of the after-nystagmus does not occur, the residue persists for days of practice rotation and instead of being further reduced, it may actually increase.

The graphs of rabbits 84 and 85 are illustrative of this. Rabbit 82 was rotated until there was no longer any measurable after-nystagmus on turning to the left. This state had been reached at the end of the fourth, fifth and sixth days of practice rotation. But at the end of the seventh day of practice rotation eight nystagmic jerks were recorded.

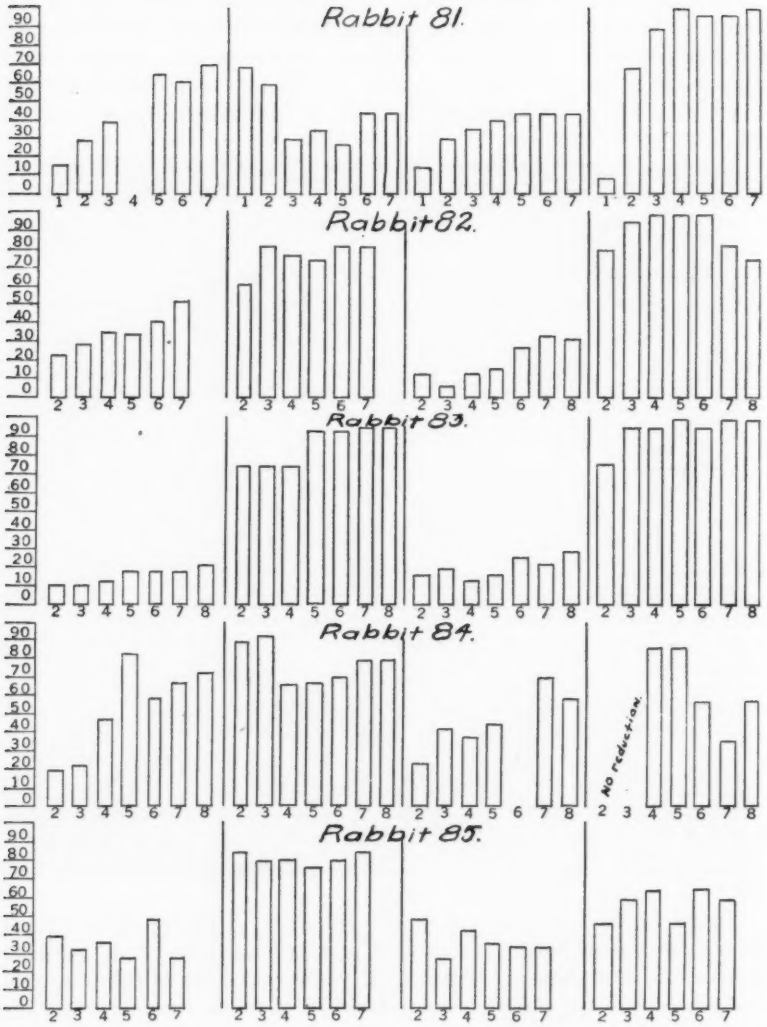

Fig. 5. Percentage reductions: ordinates. Responses at the end of each day of practice rotation: abscissae. First column, rotation-nystagmus, right. Second after-nystagmus, right. Third column, rotation-nystagmus, left. Fourth column, after nystagmus, left.

It is necessary at this point to call attention to the fact that in this paper the duration of the labyrinthine excitation is expressed in terms of number of nystagmic jerks. Therefore, when in the above paragraph, the statements are made that the after-nystagmus is obliterated, it means only that no quick movements occur. In all these experiments, the slow phase persists to some extent and indicates that some labyrinthine effect is still present. For this reason, the duration of labyrinthine effect might be more accurately represented by the time in seconds rather than as numbers of strokes. However, the points which are discussed in this paper can be brought out just as clearly by the use of either the time or the number of eye-movements and the writer has preferred to employ the latter.

That the after-nystagmus in rabbits is subject to modification by repeated rotation has already been reported by Maxwell, Reston and Burke (1922). Maxwell (1924) published a note in which he observed that the rotation-nystagmus is never obliterated and that when the after-nystagmus if fully reduced, the reduction in the rotation-nystagmus is not more than about fifty per cent. Hoshino (1922) also observed that in rabbits the after-nystagmus is reduced by repeated rotation. Dunlap and Bentley (1919) were the first to present incontrovertible evidence of the decline of the after-nystagmus in men who had been subjected to repeated rotation. Griffith (1920 a, b), Dodge (1923) and Holsopple (1923) have confirmed and materially added to this earlier work.

Figure 6 (*a*, *b*) shows the responses of animals 81 and 84 at the beginning of the practice rotations and again after they had been subjected to daily practice rotations. In the upper tracing of rabbit 84 the rotation-nystagmus persists during the whole period that the table is in motion. At the moment of stopping, the direction of the compensatory movement is reversed. In the lower tracing of this animal, the distance from crest to crest of each nystagmic movement is great as compared with the distance in the upper tracing. This difference is due to the slowness with which the compensatory phase occurs. With progressive rotation, the compensatory phase becomes slower and slower. At the beginning of rotation, the compensatory phase cannot be distinguished from the from the return phase. Near the end of rotation, the compenstory phase is readily differentiated from the return phase. The number of eye-movements in unit time necessarily depends on the velocity with which the slow phase occurs, for in order that the quick phase can occur it is essential that the eye reach almost to the position of maximum deviation. In the upper tracing, the labyrinthine excitation is very strong, for differences in the slow and the quick phase are seen only with difficulty. In the lower tracing of rabbit 84, almost from the beginning, the quick phase can be distinguished from the slow phase and it can be inferred that the

labyrinth is less powerfully excited. Similar facts were found in all of the animals which have been studied. What the exact mechanism for the elicitation of the quick phase consists of, is still a matter of controversy. De Kleyn (1921) ascribes its origin to the midbrain.

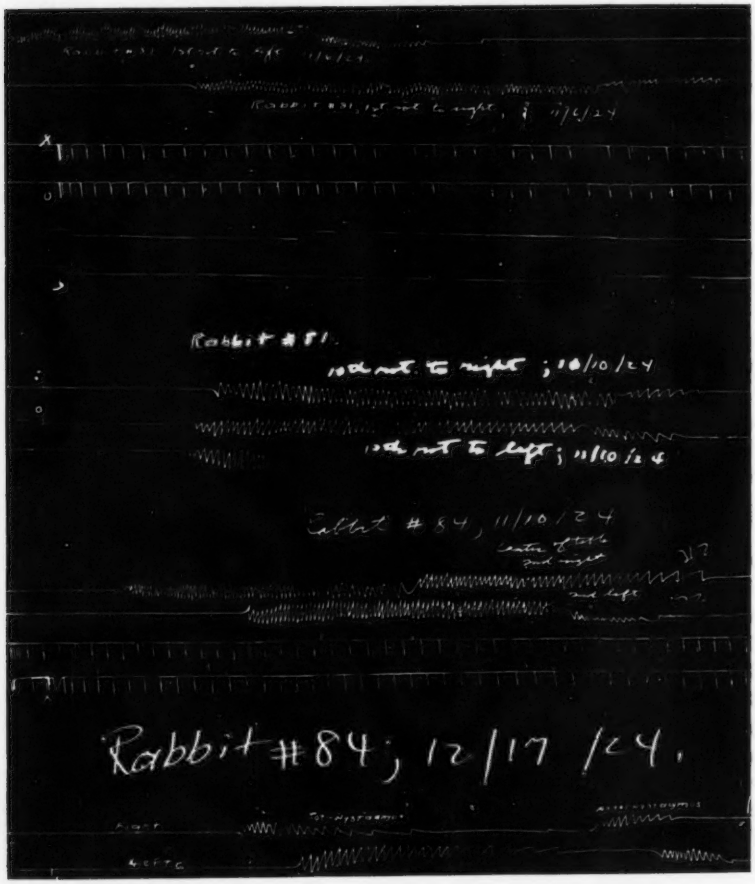

Fig. 6. Upper tracing of rabbit 81 was taken during the first rotation, whereas the lower tracing of rabbit 81 represents the responses during the twentieth rotation. Upper tracing of rabbit 84 represents the response to turning during the second rotation, whereas the lower tracing shows the response after some eight days of practice rotation.

The above is not only confirmatory evidence of the general observations of previous workers, but in addition presents some new facts. It is also

a further link in the fast accumulating mass of evidence which is rendering doubtful the value of the after-nystagmus as a functional criterion of the vestibular apparatus. In this connection most present day investigators overlook the statement of Bartels (1911) that "the failure of the after-nystagmus, with the normal, original rotation-nystagmus still occurring, shows how deceitful the employment of the after-nystagmus alone, as a test of the functional ability of the vestibular apparatus, may be." Although Bartels' statement is not quite in agreement with the work of Maxwell (1924) and of the writer, who observe that some reduction in the rotation-nystagmus occurs at the time that the after-nystagmus fails to appear, yet it does show that the limitations of this test were recognized not long after Bàràny (1907) first proposed it.

D. Recovery after practice rotation. The recovery after reduction was studied in the same five rabbits. The animals had had seven to eight days of practice rotation. Then at intervals of fifteen to thirty days, they were rotated once in order to record the ocular responses. The observations on these animals were continued from two to six months. The results of the study of the recovery of the rotation-nystagmus are not very conclusive. Some animals showed some recovery of the rotation-nystagmus while others had less response at the end of the rest period than at the end of the practice rotations. However, the inference is warranted that some recovery can occur, and further that a relatively long rest period is required. On the other hand the after-nystagmus uniformly showed after a rest period an increase over the amount occurring at the end of the practice rotation series. Contrasting the rotation-nystagmus with the after-nystagmus, it can be said on the basis of the reduction experiments and the recovery experiments that the after-nystagmus is not only more readily reduced than the rotation-nystagmus, but, moreover, the after-nystagmus recovers more quickly than the rotation-nystagmus. Griffith (1920) finds that the effects of practice rotation are still in evidence after four to eight weeks of rest. According to Holsopple (1923) after a month's rest, the recuperation of the after-nystagmus was but slight, whereas one subject showed complete recovery after seven months. Both Griffith and Holsopple used men as their experimental subjects and studied only the after-nystagmus. The writer's experiments are in accord with their work in showing that recovery of the after-nystagmus occurs to some extent, but that a long period of rest is required.

The results are given in table 3.

E. Effect of duration of rotation upon the rotation-nystagmus and the after-nystagmus. Table 4 gives the duration of the rotation-nystagmus in number of eye-movements and in number of seconds in normal rabbits rotated at a velocity of one turn in two seconds. In the experiments which are given in this table the rotation-nystagmus had ceased previous

TABLE 3

Study of the recovery of the rotation-nystagmus and of the after-nystagmus from practice effects. The data show that the effects of practice rotation are more persistent in the rotation-nystagmus than in the after-nystagmus

DATE	NUMBER OF ROTATION	ROTATION-NYSTAGMUS		AFTER-NYSTAGMUS	
		R	L	R	L
Rabbit 81					
11/ 6/24	1	90	81	20	26
11/14/24	60	34	46	11	1
11/17/24	70	26	46	11	0
	Rest period				
12/ 2/24	71	30	35	12	18
	Rest period				
12/17/24	73	22	29	7	14
	Rest period				
2/27/25	74	17	20	9	11
	Rest period				
3/11/25	80	19	36	12	15
Rabbit 82					
11/10/24	1	77	63	58	46
11/17/24	61	45	50	10	0
11/18/24	71	36	45	10	8
	Rest period				
12/ 2/24	72	22	37	11	10
	Rest period				
12/17/24	73	19	25	11	12
	Rest period				
2/27/25	74	29	37	20	15
	Rest period				
6/ 8/25	75	28	36	15	12
Rabbit 83					
11/ 7/24	3	57	66	23	17
11/17/24	68	47	48	3	1
11/18/24	78	—	51	—	0
11/19/24	88	48	47	1	0
	Rest period				
12/ 2/24	89	44	44	7	3
	Rest period				
12/17/24	90	—	36	—	12
	Rest period				
2/27/25	91	45	52	13	19

TABLE 3—*Concluded*

DATE	NUMBER OF ROTATION	ROTATION-NYSTAGMUS		AFTER-NYSTAGMUS	
		R	L	R	L
Rabbit 84					
11/10/24	2	69	65	41	14
11/18/24	73	22	22	9	9
11/19/24	83	18	31	8	6
	Rest period				
12/ 2/24	84	17	18	11	12
	Rest period				
2/17/25	85	23	22	14	14
	Rest period				
3/ 6/25	88	26	29	13	13
Rabbit 85					
11/10/24	1	25	60	55	17
11/18/24	60	13	40	8	7
11/19/24	70	18	40	8	7
	Rest period				
12/ 2/24	71	10	25	8	7
	Rest period				
1/16/25	72	14	48	22	8

TABLE 4

Showing the maximal amount of response to rotation of the rotation-nystagmus and the after-nystagmus

RABBIT NUMBER	DURATION OF ROTATION IN SECONDS	ROTATION-NYSTAGMUS		AFTER-NYSTAGMUS	
		In number of eye-movements L	In seconds L	In number of eye-movements L	In seconds L
9	45	101	35	78	20
10	73	145	64	67	21
11	60	119	51	57	22
12	71	179	65	154	54
13	58	101	48	75	40
8	60	109	54	103	38
7	65	113	53	117	48

to the cessation of rotation. Consequently the figures for the rotation-nystagmus and the after-nystagmus indicate the maximal response of the animal to the given rotation rate. It is of theoretical interest to note that in some instances the rotation-nystagmus lasts for more than a minute.

In figure 7, the duration of rotation in seconds is plotted against the number of eye-movements of the rotation-nystagmus and the after-

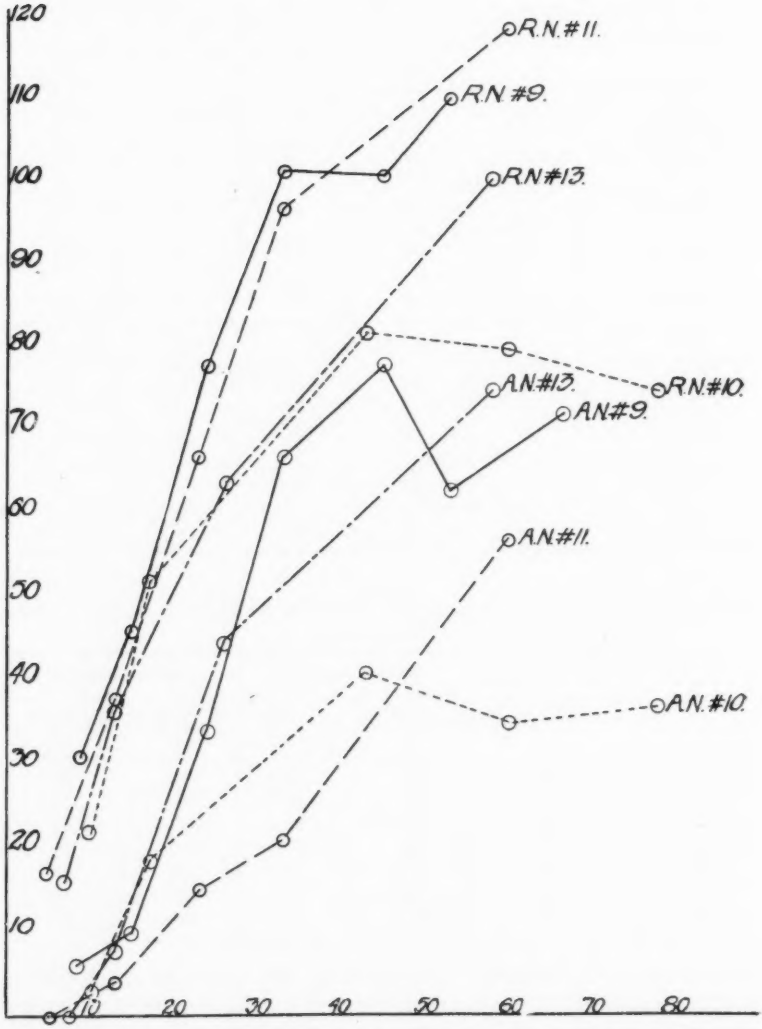

Fig. 7. Relation between duration of rotation and number of eye-movements. Ordinates: number of eye-movements of the rotation-nystagmus, *R.N.*, and of the after-nystagmus, *A.N.* Abscissae: duration of rotation in seconds.

nystagmus. It is clear that the amount of rotation-nystagmus is directly dependent upon the duration of rotation. With increasing periods of rotation up to a certain value the number of eye-movements increases. When this value, which varies with each animal, is reached further rotation does not evoke any further response.

In general the after-nystagmus behaves in a similar way, that is, with increasing durations of rotation, increasing numbers of eye-movements are elicited. The observations which are recorded in the graphs of the after-nystagmic responses of rabbits 11 and 13 are of more than passing significance. In these animals, after some seven seconds of rotation during which 15 and 16 nystagmic responses occurred, no after-nystagmus followed. When the period of rotation was increased to 12 seconds, the rotation-nystagmus was followed by an after-nystagmus.

From these observations it can be concluded that the rotation-nystagmus and the after-nystagmus are related in as much as the magnitude of the after-nystagmus is directly dependent upon the magnitude of the rotation-nystagmus.

Masuda (1922), using guinea pigs, reported that with increases in the length of the period of rotation, there occurred increases in the duration of the after-nystagmus up to a point, after which it became less with still longer periods of rotation. In the light of the writer's experiments this decrease can be explained by the fact that reduction in the after-nystagmus follows repeated rotation. A rest period of 15 minutes between successive experiments, such as Masuda allowed, which might remove the fatigue effect is not sufficient to eliminate the phenomenon of habituation. In man, Holsopple (1923) found that the after-nystagmus is greater for fifteen turns than for ten and that for some subjects it may be greater for twenty-five than for fifteen. This is in agreement with the writer's results.

SUMMARY

1. A rotation apparatus is described by means of which it is possible to record graphically the rotation-nystagmus and the after-nystagmus which occurs during and following rotation in a horizontal plane.

2. Graphic records show that the amount of rotation-nystagmus and after-nystagmus, which occur upon turning to the right and to the left, is variable and seldom are the responses the same in the two directions of turning.

3. Both rotation-nystagmus and after-nystagmus show a reduction as the result of repeated rotation. The after-nystagmus is more readily reduced than the rotation-nystagmus. Complete obliteration of the after-nystagmus has been observed repeatedly, whereas complete obliteration of the rotation-nystagmus has never been seen. The percentage reduction

both in the rotation-nystagmus and the after-nystagmus is greatest during the first two to four days of practice rotation.

4. If animals, in which the responses have been reduced by repeated rotation, are allowed rest periods, some recovery may occur. The after-nystagmus shows after the same amount of rest, a greater recovery than the rotation-nystagmus.

5. The magnitudes of the rotation-nystagmus and of the after-nystagmus are dependent upon the duration of the rotation.

BIBLIOGRAPHY

Air Service Medical, War Department, Washington, 1919.

BÁRÁNY, R. 1907. Physiologie und Pathologie des Bogengangs apparates beim Menschen. Leipzig und Wien.

DE BARENNE, D. AND DE KLEYN. 1923. Arch. f. Ophthal., cxi, 374.

BARTELS, M. 1911. Arch. f. Ophthal., lxxx, 207.

DELABARRE, E. B. 1897–98. Amer. Journ. Psychol., ix, 572.

DODGE, R. 1923. Journ. Exper. Psychol., vi, 1.

DUNLAP, K. 1919. Journ. Amer. Med. Assoc., lxxiii, 54.

GRIFFITH, C. R. 1920. Journ. Exper. Psychol., iii, 15; Amer. Nat., liv, 524.

HOLSOPPLE, J. Q. 1923. Journ. Comp. Psychol., iii, 85.

HOSHINO, T. 1922. Acta-oto., Laryngol., iv, 328.

DE KLEYN, A. 1921. K. Skad. V. Wetenschappen, Amsterdam, xxiii, 1357.

MASUDA, T. 1922. Pflüger's Arch., cxcvii, 1.

MAXWELL, S. S. 1924. This Journal, lxviii, 125.

MAXWELL, S. S., U. BURKE AND C. RESTON. 1922. This Journal, lviii, 432.

ON THE RELATION OF AFTER-NYSTAGMUS TO ROTATION-NYSTAGMUS

II. Modifying Influences

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A. Relation between the rotation-nystagmus and the after-nystagmus. Among investigators of labyrinthine function, there is the opinion that the rotation-nystagmus in the one direction of rotation is related to the after-nystagmus in the other. The fact that the directions of the compensatory phases are the same in the rotation-nystagmus following rotation in one direction and in the after-nystagmus following rotation in the opposite direction is the foundation of the belief in this reciprocal relationship.

There are some experimental observations to support this belief. Loeb and Koranyi (1891) state that, in the rabbit, after extirpation of the left cerebral hemisphere, the nystagmus during rotation to the left is greater than the nystagmus during rotation to the right, while, on the other hand the after-nystagmus to the right is greater than to the left. Whether or not normal animals react in the same manner, they do not state. Moreover, these authors present no data to support the above statement. Bartels (1911) presents another type of evidence. He recorded simultaneously the movements of both eyes. Upon rotation to the right about an arc of 180 degrees, the amplitude of the nystagmic jerks was distinctly greater in the tracing of the right eye than of the left; on the other hand, the amplitude of the after-nystagmic jerk of the left eye was greater than that of the right eye. Bartels did not place much emphasis on this reciprocal relationship except to note that it occurred, in so far as the amplitude of the movements was concerned.

The means by which the eye-movements are graphically recorded has been described in Part I of this contribution.

a. Evidence of a reciprocal relationship in normal animals. Graphic records were made of the responses to the first or second rotations of eighty animals. Some of the animals were given ten turns to each rotation; others twenty; and still others were rotated until the rotation-nystagmus had ceased. Since the purpose of this study is to determine whether or not there is any connection between the rotation-nustagmus in the one

TABLE 1

Amount of response, in the two directions of rotation, of the rotation-nystagmus and the after-nystagmus during the first rotation and in some instances during the second rotation

NUMBER OF TURNS TO EACH ROTATION	NUMBER OF ROTATION	ROTATION-NYSTAGMUS		AFTER-NYSTAGMUS	
		R	L	R	L
10	2	49	62	38	9
10	1	72	60	23	38
10	1	29	46	73	45
10	2	28	51	73	50
10	1	37	61	49	102
10	2	73	61	66	46
10	1	54	80	129	20
10	1	45	97	93	28
10	2	39	63	25	10
10	2	27	64	29	3
10	1	46	75	41	14
10	2	50	66	38	50
10	4	56	31	14	24
10	2	31	71	28	18
10	1	65	29	21	36
10	1	77	63	58	46
10	1	25	60	55	17
10	2	81	62	24	34
20	1	98	110	89	50
20	1	137	158	108	39
20	2	55	129	85	30
20	1	140	159	97	28
20	1	166	156	61	16
20	1	161	137	84	94
20	2	200	137	43	105
20	1	70	128	38	26
20	1	143	153	27	16
20	1	169	147	47	8
20	1	105	80	23	33
20	1	105	81	23	33
20	1	158	147	51	23
20	1	142	123	134	28
20	1	161	143	62	44
20	1	88	128	79	52
20	1	128	165	136	38
20	1	174	123	50	19
10	1	31	76	29	17
20	1	152	169	154	18
20	1	143	106	92	113
20	1	133	91	74	98
20	1	97	75	65	76
30 or more	1	63	145	67	96
30	1	140	159	110	46
30	1	137	158	108	39

direction of turning and the after-nystagmus following rotation in the opposite direction, only those records which showed a distinct difference between the two directions of rotation were employed. These results of forty-four animals are presented in table 1. In 33 of the 44 animals it is seen that whenever the rotation-nystagmus in one direction of turning is greater than in the other, the relations are reversed in the after-nystagmus. For example, if the rotation-nystagmus to the right is greater than to the left, then in the after-nystagmus the responses to the right are less than to the left. While this has not been found to be true for all animals, in six more of these animals in which the relation did not at first appear, it came out after a few practice rotations.

In spite of the admittedly numerous exceptions, it may still be inferred from these experiments on apparently normal animals that the rotation-nystagmus in the one direction of turning is related to the after-nystagmus in the opposite direction.

TABLE 2

Response to rotation of rabbit C-4, which showed a pathological rotation-nystagmus during turning to the left and a pathological after-nystagmus following turning to the right

Unless otherwise designated, the figures represent numbers of nystagmic movements.

DATE	NUMBER OF ROTATIONS	ROTATION-NYSTAGMUS		AFTER-NYSTAGMUS	
		R	L	R	L
7/30/24	1	174	123	50	19
7/21/24	1	82	8.5 *mins.*	4 *mins.*	4

b. Comparison of rotation responses to the right and to the left in an abnormal animal. Another line of evidence for the view that the after-nystagmus in one direction of rotation is related to the rotation-nystagmus in the opposite direction is seen in occasional abnormal animals. The protocol of rabbit C-4 may serve as an example. On the second day of rotation of this animal a very long after-nystagmus was noticed following the turning to the right. There is no doubt that this represented a pathological condition. The after-nystagmus in the other direction of rotation was normal. If the reciprocal relationship exists, then in this animal one should expect a pathological response, or at any rate a rotation-nystagmus of long duration in the opposite direction of rotation. This actually occurred, for when the animal was rotated to the left, rotation-nystagmus continued during the whole time of the turning, even when this was prolonged to eight minutes, while the after-nystagmus was composed of only four strokes. During rotation in the opposite direction, the rotation-nystagmus was normal, but the after-nystagmus lasted four minutes.

TABLE 3

Showing the effects of the removal of a posterior portion of one cerebral hemisphere on the rotation-nystagmus and the after-nystagmus. Twenty turns to each rotation

DATE	NUMBER OF ROTATION	ROTATION-NYSTAGMUS		AFTER-NYSTAGMUS	
		R	L	R	L
Rabbit A-7					
5/20/25	1	161	137	34	94*
6/20/25	Posterior portion of the right cerebral hemisphere removed				
6/27/25	Animal in good condition				
	1	173	52	15	157
	2	165	54	8	122
7/ 9/25	1	40	152	154	8
	2	46	137	239	6
7/17/25	1	85	64	32	52
	2	88	72	31	42
Autopsy: Injury included occipital, temporal and parietal areas					
Rabbit B-3					
6/15/25	1	166	156	61	19*
6/15/25	Left cerebral hemisphere in occipital region injured				
6/18/25	1	92	73	85	105
	2	93	75	63	104
Autopsy: Injury in parietal, temporal and occipital regions					
Rabbit A-5					
5/12/25	1	52	83	39	15
5/12/25	Right cerebral hemisphere injured in parietal and occipital regions				
5/14/25	1	43	51	22	9
	2	29	44	9	9
6/20/25	1	56	29	21	51
7/ 9/25	1	124	45	28	95
7/16/25	1	60	11	13	34
Post mortem: Injuries limited to parietal, temporal and occipital lobes					
Rabbit B-7					
6/28/25	1	169	147	47	8
6/28/25	2	162	145	39	4
6/28/25	Left cerebral hemisphere injured in occipital region				
7/ 6/25	1	149	141	51	18
7/ 8/25	1	154	132	19	39
Post mortem: Injury in occipital, parietal and temporal areas					

* Unoperated.

c. Evidence from animals with cerebral injuries. The statement of Loeb and Koranyi (1891) cited above is suggestive, but gives no data relative to this problem. It was deemed advisable to repeat their experiments

and to record graphically the nystagmus resulting from rotation of the operated animals.

The protocol of rabbit A-7 illustrates the connection between the rotation-nystagmus and the after-nystagmus. The operation consisted of the removal of portions of the occipital, temporal and parietal lobes of the right cerebral hemisphere. In the unoperated animal, when the rotation is to the right, the rotation-nystagmus is greater than when the rotation is to the left, while in the after-nystagmus the magnitudes of the responses are reversed. One week after the operation, there is a large increase in the after-nystagmus to the left. This confirms the work of Loeb and Koranyi (1891) and of Bauer and Leidler (1911), who found that, after removal of the right cerebral hemosphere, there is a greater after-nystagmus to the left than to the right. It is now seen that the rotation-nystagmus behaves in the opposite sense, for the response to the left is greatly decreased while to the right it is slightly greater than in the normal animal. Twelve days later the animal shows a different response; the after-nystagmus to the right is very much greater than to the left and the rotation-nystagmus to the left is now much greater than it was twelve days before. After eight days more, the responses to the two directions of rotation appear normal, but with the reciprocal relationship still in evidence.

The responses to rotation of four animals before the operation and again after a posterior portion of one cerebral hemisphere was removed are presented in table 3. Even in the cases of rabbits B-3 and B-7 which did not show the inverse relationship before the operation, it came out after the operation.

The results, given in table 3, uniformly show that whenever the after-nystagmus in the one direction of turning is affected by cerebral injury, the rotation-nystagmus in the opposite direction of rotation is simultaneously influenced. This is a quantitative confirmation of the statements of Loeb and Koranyi (1891).

Whether or not the cerebral hemispheres exert a direct influence upon nystagmus is still an undecided problem. Loeb and Koranyi (1891) and Bauer and Leidler (1911) have independently observed that if, for example, the right cerebral hemisphere is extirpated, the after-nystagmus following rotation to the left is greater than that following rotation to the right. De Barenne and de Kleyn (1923) have repeated the experiments of the above workers and discovered among other things that 1, under controlled conditions, the differences in the responses of animals, immediately after the operation, to turning in the two directions are not greater than the differences which occur in normal animals, and 2, asymmetrical responses to the two directions of turning may occur and in the same sense as Loeb and Koranyi had observed, but that such responses

are not apparent immediately after the operation, but only after the lapse of a few days. De Barenne and de Kleyn do not positively assert that the cerebral hemispheres are not concerned with nystagmus, but merely point out the precautions which are necessary in a research of this sort and the need for accurate, histologically controlled experiments.

From the writer's experiments it is seen in the cases of rabbits A-5 and B-7 that asymmetrical after-nystagmic responses do not occur until some days after the operation, thus confirming de Barenne and de Kleyn (1923). For rabbit B-7, this interval was about ten days and the response to rotation on this day was not in the sense of Loeb and Koranyi, since the after-nystagmus was greater following rotation to the left than to the right. The responses of the remaining three animals are in keeping with the observations of Loeb and Koranyi, and Bauer and Leidler. The phenomenon of reversal of response which rabbit A-7 showed, was also observed in one animal by Loeb and Koranyi (cited above).

From the above the conclusion is warranted that in rabbits with lesions of one cerebral hemisphere, if asymmetrical responses to the two directions of turning occur, there is exhibited a reciprocal relationship between the rotation nystagmus and the after-nystagmus.

d. Evidence from the destruction of one labyrinth. Experiments on the destruction of one labyrinth were pursued with a twofold purpose; first, to confirm the work of previous investigators, and second, to note whether the rotation responses of animals with one intact labyrinth would shed any light on the question of the reciprocal relationship between the rotation-nystagmus and the after-nystagmus.

Confirmation of the older work seemed highly desirable, since the conclusions drawn from the rotation responses of guinea pigs and rabbits with one intact labyrinth are at variance with one another. According to Bartels (1910), after extirpation of one labyrinth in the rabbit, the rotation-nystagmus as well as the after-nystagmus is very small or fails entirely when the rotation is towards the injured side. But in his protocols, the results do not appear to warrant any such conclusion. For example, after section of the left eighth nerve of rabbit 94, he finds that upon rotation to the left, a few nystagmic jerks occur and fifteen or twenty after-nystagmic movements; during rotation to the right, distinct nystagmic jerks occur, followed on cessation of rotation by two slow jerks. The writer would conclude from data of this sort that destruction of one labyrinth affects the nystagmus towards the injured side and the after-nystagmus upon rotation towards the uninjured side. Using guinea pigs, Dreyfuss (1900) noted, after one-sided labyrinthine injury, that both rotation-nystagmus and after-nystagmus occur whether the turning is towards the injured or the uninjured side. No figures are given as to the actual amounts. Detlefson (1925) reports that in rats when the left

labyrinth is injured, the after-nystagmus following counter-clockwise rotation is greater than the after-nystagmus following clockwise rotation. When the right labyrinth is destroyed, the after-nystagmus after clockwise rotation is greater than after counter-clockwise turning. This is strong evidence for the existence of the reciprocal relationship in the rat. Trendelenburg and Kühn (1908), employing lizards and other reptiles, found, after one-sided labyrinthine destruction and with the eyes of the animals closed, that rotation-nystagmus of the head is not present when the turning is toward the injured side, but there is a normal after-nystagmus; rotation-nystagmus occurs upon turning toward the uninjured side, but no after-nystagmus is visible. These results coincide neither with the work on rabbits nor with the observations on guinea pigs. In the dogfish, Maxwell (1919 and 1923) found that when the right labyrinth is destroyed, upon rotation to the left in a horizontal plane, the two eyes make a conjugate movement to the right. When the animal is rotated to the right, there is no reaction of the eyes. This observation is in agreement with that of Trendelenburg and Kühn on the reptile.

In the following experiments the labyrinth was destroyed by the insertion of a steel probe through the external auditory meatus, penetrating the fenestra ovale and entering the vestibule. In all cases the injuries were histologically controlled. The slumping of the head towards the side of injury, the spontaneous nystagmic movements of the head and eyes and the rolling movements of the animal were employed as external criteria of labyrinthine injury. The operated animals were not rotated until the disappearance of spontaneous nystagmus. In all experiments, the eye movements were recorded graphically. The animals had not been previously rotated. Each rotation consisted of twenty turns.

The results obtained after destruction of the right labyrinth of rabbit C-2 are typical. In this animal upon rotation to the right, a small amount of rotation-nystagmus is present and the after-nystagmus appears normal. Upon rotation to the left, rotation-nystagmus is present, but there is only a small amount of after-nystagmus.

Five experiments are given in table 4.

The tracing of the responses of rabbit C-3, in which the left labyrinth had been destroyed, is shown in figure 1. Upon rotation to the left, there are five movements which have the appearance of a typical nystagmus. Some nystagmus is present during the whole of the rotation, but the direction of the slow phase of this nystagmus is opposite to that of the first five nystagmic jerks and is the same as the direction of the slow phase during the rotation to the right. A typical after-nystagmus occurs; the direction of the slow phase of this after-nystagmus is the same as the direction of the slow phase of the nystagmus which occurred, following the first five nystagmic jerks. Upon rotation to the right, a typical ro-

tation-nystagmus occurs, while the after-nystagmus is represented by three jerks.

In these experiments after destruction of one labyrinth both rotation-nystagmus and after-nystagmus occur upon rotation to the injured as well

TABLE 4

Showing the rotation responses to the right and to the left of animals, in which one labyrinth has been destroyed

DATE	ROTATION-NYSTAGMUS		AFTER-NYSTAGMUS	
	R	L	R	L
Rabbit C-3				
8/ 7/25	122	128	94	11
8/ 7/25	Left labyrinth destroyed. Head slumps to left. Spontaneous nystagmus of head and eyes is present. Rolling movements of body.			
8/ 9/25	Head bent to left. Some spontaneous nystagmus still present			
8/ 9/25	Typical nystagmus	5	2	21
Rabbit C-2				
7/29/25	67	131	94	5
8/ 3/25	Right labyrinth destroyed. Spontaneous nystagmus present. Head tilted to right			
8/ 5/25	3	30	30	2
Rabbit C-1				
8/21/25	55	36	20	12
8/21/25	Right labyrinth destroyed			
8/25/25	4	14	13	4
Rabbit C-8				
9/10/25	133	144	130	101
9/10/25	Right labyrinth destroyed			
9/21/25	5	43	27	3
Rabbit A-8				
9/10/25	127	129	72	30
9/10/25	Right labyrinth destroyed			
9/24/25	3	12	17	5

Tracings of the eye-movements of A-8 are given in figure 2.

as to the uninjured sides, thus agreeing with the results of Dreyfuss (1900) on the guinea pig. Furthermore, after extirpation of one labyrinth, less rotation-nystagmus occurs upon rotation towards the injured, than upon turning towards the uninjured side. The behavior of the after-nystagmus

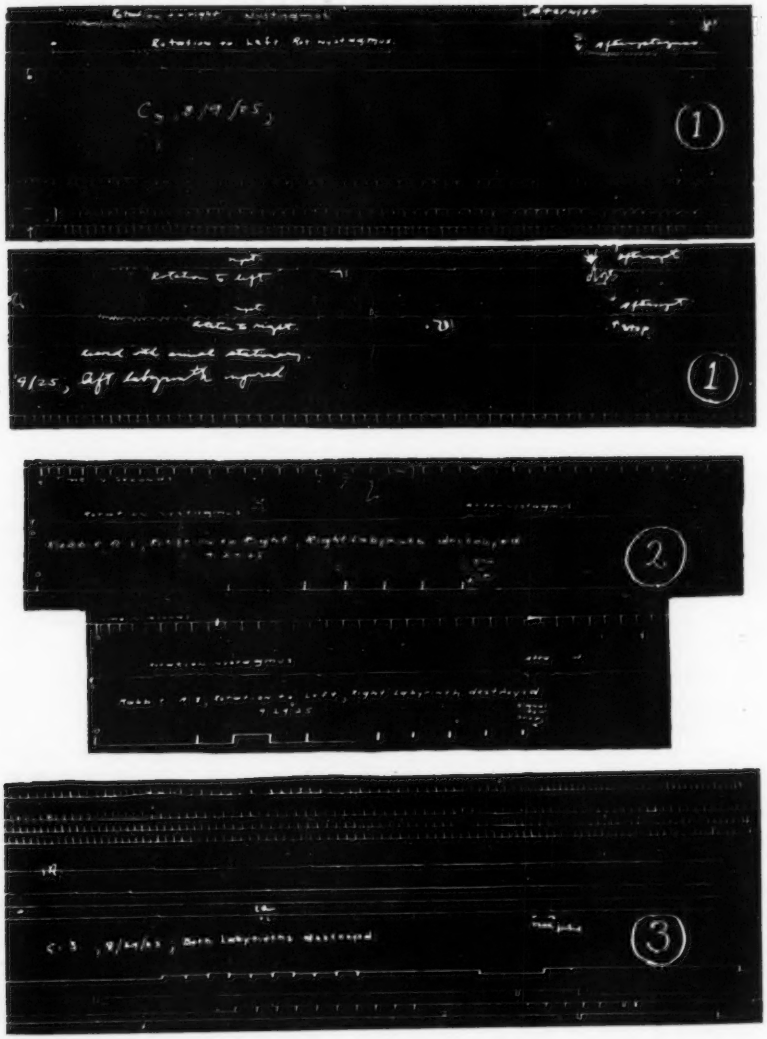

Fig. 1. Tracing of rabbit C-3, showing response to clockwise and counter-clockwise rotation, after destruction of the left labyrinth.

The lower tracing was obtained while the animal was still exhibiting spontaneous nystagmus.

Fig. 2. Response to rotation, clockwise and counter-clockwise, of rabbit A-8, after destruction of the right labyrinth.

Fig. 3. Response to rotation, clockwise and counter-clockwise, of rabbit C-3, after destruction of both labyrinths.

is the reverse, the after-nystagmus toward the injured side being greater than when the direction of turning is toward the uninjured side. It is also evident from the data that the rotation-nystagmus towards the uninjured side and the after-nystagmus toward the injured side are much smaller in magnitude than in the animal with both labyrinths intact.

In view of the experiments of Trendelenburg and Kühn (1908) in one sided labyrinthine extirpations in lizards and reptiles with open and closed eyes, the question of optical reflexes assumes importance. These workers noted rotation-nystagmus when the turning is toward the injured side and the eyes of the animal are open. The rotation-nystagmus disappears when the eyes are closed. In the following data from rabbits A-6 and A-8, by rotating the animals with the eyes covered and uncovered, an answer was sought to this query.

Rabbit A-6; Left labyrinth destroyed

DATE	NUMBER OF ROTATIONS	ROTATION-NYSTAGMUS		AFTER-NYSTAGMUS		
		R	L	R	L	
9/2/25	1	8	7	5	13	Eyes uncovered
9/2/25	2	2	7	9	28	Eyes covered
	3	19	9	4	10	Eyes uncovered

Rabbit A-8; Right labyrinth destroyed

DATE	NUMBER OF ROTATIONS	ROTATION-NYSTAGMUS		AFTER-NYSTAGMUS		
		R	L	R	L	
9/2/25	1	5	11	10	5	Eyes uncovered
	2	6	10	10	5	Eyes uncovered
	3	8	9	11	8	Eyes covered
	4	7	18	12	5	Eyes uncovered

When the eyes are covered, rotation-nystagmus and after-nystagmus still occur. It can, therefore, be said that the residual rotation-nystagmus occurring after rotation toward the injured side and the after-nystagmus toward the opposite side are not optical in character.

The above described results show that in those animals in which responses to rotations both to the right and to the left are retained after destruction of one labyrinth, a reciprocal relation between the rotation-nystagmus and the after-nystagmus definitely exists. Since the same phenomenon may be seen in normal animals and in animals with cerebral injuries it may be positively affirmed that in the normal functioning of the labyrinth such a relation exists. The nature of this relation will be discussed in another section.

B. NYSTAGMIC EYE-MOVEMENTS AFTER DESTRUCTION OF BOTH LABYRINTHS. It is known that occasional eye-movements occur when rabbits are rotated after destruction of both labyrinths. The few movements which are seen in rabbits, with one labyrinth destroyed, when rotated toward the injured side, might be of the same nature and not of labyrinthine origin. For this reason it is necessary to examine the reactions to rotation of labyrinthless animals. Rabbits C-2 and C-3, whose reactions after loss of one labyrinth were described in the preceding section, were employed for this purpose. In each of these the remaining labyrinth was destroyed and the effects of rotation were examined on the second day and on the fifth day after the operation. On the second day after the operation, spontaneous nystagmus was present; the direction of the slow phase of this nystagmus was the same during the rotation as upon cessation of rotation. Rotation, clockwise or counter-clockwise, did not affect the direction of the slow phase. Five days after the operation, spontaneous nystagmus was not present. Upon rotation to the right or to the left, one or two nystagmic jerks occurred. The after-nystagmus consisted of two or three jerks. Both in the rotation-nystagmus and in the after-nystagmus, to the right and to the left, the directions of the slow phases were the same. Covering the eyes caused no alterations of these movements. The difference between the eye-movements which occur in the animals with both labyrinths destroyed and in those toward the side of injury when one labyrinth is destroyed, is very striking. Whereas in the rotation-nystagmus toward the injured side of animals with one intact labyrinth, the jerks are three, four, or more in number and opposite in character to the rotation-nystagmus toward the uninjured side, in the animals with both labyrinths removed only one or two nystagmic strokes occur and they are spread apart and independent of the direction of rotation (fig. 3). It is conceivable that these eye-movements which occur in animals without labyrinths are secondary effects due to some slight movement of the head at the start and at the cessation of rotation, or else that they are due to the Bárány effect. Bárány (1906) demonstrated the fact that if the head of a rabbit is fixed, so that the labyrinthine effect is excluded, bending of the body to the right causes deviation of the eyes to the left. It is possible that inertia effects upon the body and head may produce analogous tension or pressure changes in the muscles or joints sufficient to excite eye-movements reflexly. Whatever the cause of these movements may be, a careful comparison shows that they do not at all correspond to the nystagmus which arises when the rabbit with one labyrinth extirpated is rotated toward the injured side.

C. RÔLE OF THE RETINA IN THE MODIFICATION OF LABYRINTHINE REACTIONS. Whether or not in the rabbit the retina is capable of influencing the labyrinthine reactions is a matter of dispute. Bartels (1920) states

that the rabbit does not show optical nystagmus. According to Hoshino (1922) no difference is found in the after-nystagmus of rabbits with covered and uncovered eyes. Magnus and de Kleyn (1921) state that in the rabbit the retina plays but a small rôle in the maintenance of body posture. On the other hand, Maxwell and Pilz (1924) have shown that the rotation-nystagmus is subject to the influence of light. In the present paper it will be shown that the labyrinthine after-nystagmus as well as the labyrinthine rotation-nystagmus can be modified by retinal stimulation.

The writer has repeatedly observed that rabbits show optical nystagmus when a cylinder of vertical, alternate black and white stripes is rotated about them. In practice, a cylinder, 72 cm. in height and 60 cm. in width provided with a stationary platform, located at the bottom of the cylinder was employed. The cylinder was electrically propelled. The white stripes were 7 cm. in width and 8 cm. apart. After the animal was placed on the stationary platform no optical nystagmus could be observed until the cylinder had been smoothly rotating for some seconds. This observation may account for the negative findings of other workers. After the cylinder has been rotating for some few seconds both head and eye nystagmus occur. The head-nystagmus can be eliminated by fixing the head in a Czermak head-holder. In these experiments the velocity of rotation was one revolution in seven to nine seconds.

While the above type of experiment shows that optical nystagmus does occur, nevertheless it gives no information regarding its relationship to the labyrinthine nystagmus.

The ordinary method of attacking the problem of the relationship of the labyrinthine nystagmus to the optical nystagmus is to observe the differences in response which are obtained when the eyes are open and when they are closed. But in the rabbit, because the labyrinthine responses are modified by repeated rotation, such differences are not readily demonstrable. Moreover, there is even a difference in response between the first rotation and the second rotation, as was pointed out in a previous paper. Further, it was shown that the after-nystagmus is extremely susceptible to modification by repeated rotation, and indeed, it is more readily modified than the rotation-nystagmus. This latter observation is evidently the reason why Maxwell and Pilz (1924) were able to demonstrate the rôle of retinal excitations on the rotation-nystagmus, but omitted any discussion of the effect of the retina on the after-nystagmus.

In view of the above, it is necessary to employ animals in which the changes from rotation to rotation are at a minimum. For this reason animals 81, 82 and 84, which had been subjected to some seven or eight days of practice rotation were used. The amount of after-nystagmus which these animals showed was small and varied but slightly from rotation to rotation. In section C, it has already been commented upon

that after a few days of practice rotation, the rotation-nystagmus as well as the after-nystagmus reach a point from which they do not greatly vary upon subsequent rotation.

TABLE 5

Showing the effect of the retina on rotation-nystagmus and after-nystagmus. Ten turns to each rotation

NUMBER OF ROTATION	ROTATION-NYSTAGMUS		AFTER-NYSTAGMUS		REMARKS
	R	L	R	L	
			Rabbit 81		
1	17	20	9	11	Eyes uncovered
2	14	14	11	18	Eyes covered
3	18	19	9	11	Eyes uncovered
1	19	36	12	15	Eyes uncovered
2	24	42	10	14	Eyes uncovered
3	20	19	14	19	Eyes covered
4	23	43	8	13	Eyes uncovered
			Rabbit 83		
1	45	45	14	16	Eyes uncovered
2	42	27	14	16	Eyes covered
1	34	26	9	15	Eyes uncovered
2	30		10		Eyes uncovered
3	33	20	12	19	Eyes covered
4	30	20	13	20	Eyes covered
5	34	27	10	11	Eyes uncovered
1	34	21	10	19	Eyes uncovered
2	31	24	12	17	Eyes uncovered
3	30	19	17	22	Eyes covered
4	32	26	12	17	Eyes uncovered
			Rabbit 84		
1	26	29	13	13	Eyes uncovered
2	23	30	17	17	Eyes uncovered
3	26	22	11	28	Eyes covered
4	30	30	11	18	Eyes uncovered
1	17	25	9	8	Eyes uncovered
2	15	26	11	11	Eyes uncovered
3	14	13	10	10	Eyes covered
4	15	12	10	11	Eyes covered
5	14	29	9	9	Eyes uncovered

The experiments were conducted as follows: A normal record of such habituated animals was taken. Hoods were placed on the eyes and a second record taken. The hoods were removed, and a third tracing

recorded. The results are presented in table 5. From these data it is evident that in the majority of cases less rotation-nystagmus occurs when the eyes are covered than when they are open, and on the other hand that more after-nystagmus occurs when the eyes are closed than when they are open. This is in agreement with the observations of Loeb (1907) on Phrynosoma. The differences which have been found between the responses with the uncovered eyes and with the covered eyes, although small, are nevertheless distinct. Bearing in mind the fact that Hoshino employed the method of inspection and that he did not take into account the fact that the after-nystagmus is so extremely subject to modification, it is clear why he concluded that in the rabbit the retinal effect was not present.

The explanation of the rôle of the retina as advanced by Loeb (1907) is applicable to the rabbit, namely, that during rotation, the effect of retinal stimulation is in the same direction as that of the labyrinthine stimulation. The rotation-nystagmus is, therefore, the algebraic sum of two positive quantities. Upon cessation of rotation, the retinal stimulation continues in the same direction, whereas the labyrinthine after-effect is reversed. The after-nystagmus, consequently, is equal to the sum of the quantities of opposite sign. The above explanation holds for animals with the eyes open. With closed eyes, there is no retinal effect, and consequently the rotation-nystagmus is less than with the open eyes, but the after-nystagmus is greater, since the counteracting effect of the retina is lacking.

In view of the above findings it is concluded that there is retinal modification of labyrinthine effects in the rabbit, but of such low magnitude that special measures must be employed to demonstrate it.

Concluding remarks. The facts presented in the foregoing sections give evidence that in the rabbit each labyrinth has the ability to respond to rotational movements in both directions, but in unequal amounts. In other words, each labyrinth functions for both sides. It may be assumed that in the normal animal upon rotation to the right, stimulation of the receptor structures in the right labyrinth accounts for the major part of the rotation-nystagmus, while the remainder of the effect is due to the stimulation of the left labyrinth. On the other hand, the after-nystagmus following rotation to the right is chiefly due to excitations arising from stimulation of the left labyrinth and to a lesser extent of the right labyrinth, since more after-nystagmus occurs upon rotation to the right than upon rotation to the left, when the right labyrinth has been destroyed. To account for the fact that one labyrinth causes rotation-nystagmus, both to the right and to the left, it must be assumed that in the nervous pathway between the receptors in the labyrinth and the eye-muscle nuclei, there occur connections both with the ipsilateral and

the contralateral sides. The number of fibers in the crossed connection is apparently small as compared with the number of undecussated fibers.

An examination of the literature of labyrinth extirpation and of section of the eighth nerve shows that the rabbit and the dogfish represent two general types. It appears that in the fishes, amphibians, and reptiles, each labyrinth functions only for rotations towards its own side, while in birds and mammals each labyrinth gives in addition a lesser response to rotation to the opposite side. It is interesting to compare these relations, unilateral or bilateral, of the equilibrial receptors in the ear, with the analogous relations of the receptors for light. One thinks of the complete decussation in the fishes and the partial decussation in the mammal.

SUMMARY

1. In the majority of normal animals in which there is a distinct difference in the responses in the two directions of rotation, a reciprocal relation is seen between the rotation-nystagmus on turning in one direction and the after-nystagmus in the opposite direction of turning. Thus, if the rotation-nystagmus, following turning to the right is greater than that to the left, the after-nystagmus to the left is greater than that to the right.

2. In animals with asymmetrical cerebral lesions, if the after-nystagmus following turning in the one direction is affected, the rotation-nystagmus in the opposite direction of turning is also affected.

3. After destruction of one labyrinth, rotation-nystagmus occurs upon turning to the injured as well as to the uninjured side. There is more rotation-nystagmus and less after-nystagmus upon rotation to the uninjured side than upon rotation toward the injured side and vice versa.

4. Contrary to the statements of previous observers, nystagmus can be induced in the rabbit by a moving retinal field.

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BIBLIOGRAPHY

Bauer, J. and R. Leidler. 1911. Arb. Neurol. Inst. Wien, xix, 155.
Barany, R. 1906. Zentralbl. f. Physiol., xx, 298.
De Barenne, D. and A. de Kleyn. 1923. Arch. f. Ophthal., cxi, 374.
Bartels, M. 1910. Arch. f. Ophthal., lxxvi, 1.
1911. Arch. f. Ophthal., lxxx, 207.
1920. Arch. f. Ophthal., ci, 299.
Detlefsen, J. A. 1925. Physiol. Rev., v, 244.
Dreyfuss, R. 1900. Pflüger's Arch., lxxxi, 604.
Hoshino, T. 1922. Acta-Oto-Laryngol., iv, 328.

LOEB, J. AND A. KORANYI. 1891. Pflüger's Arch., xlviii, 423.
LOEB, J. 1907. Pflüger's Arch., cxvi, 368.
MAGNUS, R. AND A. DE KLEYN. 1921. Pflüger's Arch., clxxxvi, 39.
MAXWELL, S. S. 1919. Journ. Gen. Physiol., ii, 123.
1923. Labyrinth and equilibrium. Philadelphia.
MAXWELL, S. S. AND G. F. PILZ. 1924. This Journal, lxx, 118.
TRENDELENBURG, W. AND A. KÜHN. 1908. Arch. f. Anat. u. Physiol., 160.

STUDIES ON THE CARDIAC OUTPUT OF THE DOG

I. The Cardiac Output of the Normal Unanesthetized Dog[1]

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The question of the quantity of blood which is pumped by the heart per minute, the minute volume, is fundamental to many problems of the circulation in health and disease. This has been a subject of much investigation. Reviews are given by Tigerstedt (1905, 1922) and Henderson (1923). The earliest attempts to solve this question were little more than guesses based on a consideration of the size of the ventricles. After the development of methods for keeping the isolated heart or a heart-lung preparation alive by perfusion, accurate estimates of the output of such a heart could be obtained. But, although this line of investigation has added enormously to our knowledge of the heart, it tells us rather what the heart can do than what it actually does in a normal man or animal. The numerous investigations by means of some form of cardiometer or stromuhr give values for the cardiac output of the heart *in situ*. Such studies have been carried out mainly by R. Tigerstedt (1891) C. Tigerstedt (1909, 1922), Elving and v. Wendt (1907), Roy and Adami (1892), Johansson and Tigerstedt (1889, 1891) and Henderson (1906, 1909). Owing to the fact that these methods necessitate that the chest of the animal be opened and that artificial respiration be given, to say nothing of the complicating effects of an anesthetic or narcotic, it seems unjustifiable to apply the values obtained to the normal healthy unanesthetized animal.

Another method which avoids opening the chest of the animal is that first used by Hering (1829) of measuring the circulation time, the time required for blood to complete the round and return to the same point. Stewart (1894, 1921) and Bock and Bucholtz (1920) are the most recent investigators who have used a modified form of this method.

In 1870, Fick proposed a method for the measurement of the amount of blood passing through the lungs, the minute volume of the right ventricle and hence of the left ventricle. He did not apply the method probably

[1] The data of this paper were presented before the American Physiological Society at the Washington Meeting, December 1924 (This Journal, 1925, lxxii, 177) and at the Cleveland meeting, December, 1925 (This Journal, 1926, lxxvi, 178).

owing to the lack of suitable analytical methods, but this was first done on dogs by Grehant and Quinquaud (1886). They merely give the calculated values varying from 512 to 2614 cc. for dogs of 7 to 18 kilos. The Fick principle, as it has come to be called, consists in determining the total quantities of oxygen or carbon dioxide in the blood of the right and left ventricles, and simultaneously estimating the quantity of oxygen absorbed or carbon dioxide given off by the animal through the lungs. Then if $M.\ V.$ represent the minute volume of blood in litres discharged by one ventricle, A_o, the quantity of oxygen and A_c the quantity of carbon dioxide in the arterial blood, V_o the amount of oxygen and V_c the amount of carbon dioxide in the mixed venous blood (all expressed in cubic centimeters per litre of blood) O the oxygen absorbed and C the carbon dioxide given off in cubic centimeter per minute, we have

$$M.V. = \frac{O}{A_o - V_o} = \frac{C}{V_c - A_c}$$

If we do not consider the now discredited view once held by Bohr (Bohr and Henriques, 1897; Starling and Evans, 1913; Hill, 1913) and others that the lungs are the seat of a considerable oxidation, the theory of the method would appear unimpeachable. Technical difficulties have, however, apparently prevented its very wide application, and frequently detract from its accuracy. It appears to be generally agreed that the figures obtained from the oxygen values are more reliable than those from the carbon dioxide results.

A few investigators have used this method on anesthetized animals, although their work has been quite limited in character. The work of Murlin and Greer (1914), Doi (1921), Uyeno (1922), Harrison, Dock and Holman (1924) and Huggett (1924) may be mentioned. The classical investigations by this method are those of Zuntz and Hagemann (1898) on the horse during rest and work. These were done without anesthesia and with only the slight operations which were necessary to introduce a sound into the jugular vein and to cannulate an artery. Barcroft, Boycott, Dunn and Peters (1919) succeeded in carrying out experiments on normal unanesthetized, unoperated goats. They determined the oxygen consumption by use of a special mask and obtained the arterial and mixed venous blood by puncture of the left and right ventricles with a syringe and needle. Odaira (1925) has quite recently reported experiments on unanesthetized rabbits studying the effect of the glands of internal secretion on the minute volume.

A great many investigators have attempted to solve the problem of the amount of blood pumped by the heart per minute by using the normal human subject. Many of the methods devised for determining the cardiac output in man have been based upon the Fick principle but the difficulty

has been in estimating the gas contents of the mixed venous blood. The gas contents of the arterial blood can be easily obtained from blood drawn by arterial puncture or indirectly by analysis of alveolar air but the determinations of the mixed venous blood must be made by some indirect method. Various schemes for doing this have been proposed by Loewy and Schrötter (1905), Plesch (1909), Douglas and Haldane (1922), Henderson and Prince (1917), Burwell and Robinson (1924), Field, Bock, Gildea and Lathrop (1924) and others. The nitrous oxid method of Krogh and Lindhard (1912) represents a different procedure. Eyster and Meek (1920) have used a method based on measurement of the x-ray shadows obtained during systole and diastole. This has also been applied to animals (Meek and Eyster, 1922). The most recent method devised for man is that of Henderson and Haggard (1925) making use of ethyl iodide.

All of the methods of determining the minute volume of the heart in man are indirect ones, and give quite varying values for the normal depending on the method. It is quite obvious that these discordant results cannot all be correct, but the difficulty lies in deciding which are the correct ones. Such an important question as the constancy of the minute volume in the same individual from time to time is answered differently by various observers. The influence on minute volume of changes in pulse rate, oxygen consumption, and ventilation has not been satisfactorily studied in the normal animal or man. A satisfactory study of these matters is necessary before much progress can be made in understanding how the circulation is controlled and the factors normally influencing it. Studies on the human subject are beset with the difficulty of being sure that the method used gives correct results. On the other hand, the Fick principle applied by direct withdrawal of arterial and mixed venous blood is theoretically sound. If applied successfully to normal unanesthetized animals the results should be accurate. The present communication describes experiments determining the minute volume of the circulation of a few normal dogs by this method over a considerable period of time. The influence of changes in pulse rate, oxygen consumption and ventilation has been determined. It is hoped that the study may may serve as a background to test existing methods or develop a satisfactory one for estimating the cardiac output in the human subject.

Methods. The Fick principle has been applied with some modifications as used by Barcroft, Boycott, Dunn and Peters (1919) on the goat. All experiments have been carried out on dogs. The results to be presented were obtained on five animals. Dogs of a friendly quiet disposition were selected. Throughout the experiments the dogs were kept in cages but frequently allowed to use a large runway. The animals have been under the care of one attendant during the whole period. They were fed at about 4 p.m. each day, the diet consisting of dog biscuit and frequently

a small amount of raw meat. Experiments were always performed before feeding and usually in the morning.

The animals lay on a table well padded with blankets. No restraint in the way of animal boards or ropes was used. In training a new animal, he was accustomed to lie on the table for periods of several minutes for two or three days. A respiration mask was made and the animal accustomed to the mask and to lying quietly on the table for about 15 minutes. A week of such training was usually necessary before an experiment could be attempted. On one or two occasions a successful experiment was completed on the third day the dog had been taken into the laboratory. Any marked movement or struggling of an animal during an experiment will vitiate the whole procedure.

The estimation of the oxygen consumption was carried out as follows: A mask was designed for me by Dr. A. C. Kolls. This mask was made for each new animal, and replaced once or twice during the investigation. Ordinary surgical plaster of paris bandage about 2 inches wide was soaked in water and wrapped repeatedly around the animal's snout. The mask was dried, and coated thoroughly inside and out with paraffin. A respiration valve of the Lòven type was sealed into the end with plastecine and the other end fitted with a collar of plastecine. This mask has a certain amount of dead space, but probably less than those of other types. The dog's head around the mouth was smeared with a solution of gum tragacanth, glycerin and water, the mask put on, and an air tight joint made by molding the plastecine around the head with the fingers. The expiratory side of the valve was attached with corrugated tubing to a small Douglas bag of 30 litres capacity. The collection of expired air was carried out in the usual way after first washing the bag. The time of collection varied with the breathing of the animal but was usually 3 to 5 minutes. The contents of the Douglas bag were at once measured by means of a carefully calibrated 3-litre wet meter, and a sample taken over mercury. The gas sample was analyzed with the Henderson modification of the Haldane apparatus. In nearly all cases duplicate determinations were made.

The dog was placed lying on his left side during the metabolism experiment. Immediately after the collection of the expired air the animal was gently turned on its back and blood collected from the right and left ventricles. A syringe containing a few cubic centimeters of paraffin oil and equipped with a 3 inch no. 20 gauge needle was used. For puncture of the left ventricle, the needle was inserted into the left costal interspace where the maximum impulse could be felt. The puncture was made almost perpendicularly downwards. The right ventricle was reached by turning the animal slightly to the right, and inserting the needle pointing inwards and towards the tail into the right costal interspace just above that used for the left ventricle 2 or 3 cm. from the sternum. The right puncture

was always made first. About 8 to 10 cc. of blood were taken from each ventricle. Samples of blood were immediately transferred to bottles containing paraffin oil and powdered potassium oxalate. Special experiments showed that no different results were obtained when the metabolism was taken with the dog on its back or the ventricular punctures made during the collection of air.

Until the technique of ventricular punctures had been thoroughly mastered, two samples of mixed venous blood were withdrawn and analyzed. It is comparatively easy to obtain left ventricular blood from the right side and vice versa, and although the color of the blood is a safe guide as to which ventricle has been punctured, another difficulty was feared. This was the possibility of obtaining a mixture of left and right heart blood from a right side puncture, and we are reasonably sure that this has happened in two or three instances. After some experience with the procedure, considerable confidence was felt in being sure of having obtained unmixed right ventricular blood. The arterial blood was occasionally obtained by puncture of the femoral artery.

In carrying out the procedures outlined above, it is essential that the dog be quiet from the commencement of the collection of expired air until the right ventricular blood has been drawn. Slight movement before drawing arterial blood is of less consequence. The experiment has frequently been put through with a change of only 3 or 4 beats in the pulse rate per minute. If one realizes the marked influence of excitement or psychic disturbances on the pulse rate of the dog, this may be taken as a guarantee that the animals were perfectly normal. No pain of any kind is connected with the punctures; the occurrence of it would spoil the whole experiment.

The samples of blood were analyzed for their total oxygen contents by the Van Slyke apparatus and technique. In the experiments until December 1924 the technique and apparatus of Van Slyke and Stadie (1921) were used, the determinations after this date were made with the constant volume apparatus and technique of Van Slyke and Neill (1924). Two cubic centimeter samples of blood were used for analysis. The oxygen was not determined by absorption, but the corrections for nitrogen used. Since it is the arterial-venous difference which is desired and not absolute values for arterial and venous oxygen, this procedure appeared just as accurate as absorption. Duplicate determinations were always done, and if agreement was not obtained a third sample was analyzed.

The animals were allowed to rest on the table for at least ten minutes and frequently two hours before the experiment. The conditions are generally not quite basal, since our experience confirms that of Kitchen (1924) that a long period (one or two hours) of rest is required to get the basal metabolism of a dog.

The pulse rate was taken two or three times a minute throughout the experiment by palpating the chest or femoral artery. In general only slight variations were noted, usually 2 to 5 beats per minute but occasionally a maximum variation of 8 to 10 beats. The average of all the figures was taken.

TABLE 1

"Brownie," male, weight on January 22, 1924, 12.0 kilos; on February 25, 14.6 kilos; March 22, 16.7 kilos; on July 8, 18.1 kilos; on January 15, 1925, 19.9 kilos; on February 20, 18.8 kilos; on February 10, 1926, 19.3 kilos. Dog was full grown, but in ill nourished and poor condition when experiments were started.

DATE	MINUTE VOLUME	PULSE PER MINUTE	OXYGEN USED PER MINUTE	RESPIRATION LITRES PER MINUTE	ARTERIAL OXYGEN	ARTERIAL VENOUS DIFFERENCE
1924	*litres*		*cc.*		*volume per cent*	*volume per cent*
January 22	2.47	160	134	3.90	16.64	5.40
January 24	2.42	112	133	4.32	16.14	5.44
January 27	2.45	112	125	3.07	16.34	5.08
January 28	2.64	100	114	3.34	13.73	4.31
February 4	2.40	90	153	3.74	14.36	6.36
February 17	2.76	100	105	2.06	14.74	3.77
February 25	2.64	109	137	3.30	15.69	5.20
February 25	3.02	101	133	3.51	15.74	4.40
March 5	2.66	94	107	3.16	13.17	4.39
March 5	2.53	84	108	3.26	12.72	4.69
March 13	2.71	83	104	2.84	13.22	3.83
March 22	2.63	92	121	2.69	14.54	4.61
July 8	2.66	73	110	3.22	13.98	4.13
July 16	2.88	74	101	3.25	14.41	3.51
July 18	2.78	81	110	2.65	14.70	3.96
1925						
January 15	2.69	87	117	3.00	20.38	4.35
January 15	2.61	76	108	3.00	20.63	4.14
February 3	2.40	69	100	3.64	18.11	4.17
February 5	2.65	86	116	3.72	17.86	4.37
February 17	2.34	74	123	4.15	18.62	5.26
February 26	2.55	69	110	4.11	17.88	4.32
March 14	2.51	80	113	5.61	21.45	4.50
October 7	2.41	84	89	2.53	21.40	3.70
1926						
February 15	2.37	91	130	5.55	21.53	5.49

The question of the ventricular punctures disturbing the heart or circulation has been frequently considered. Two of the dogs were attached to an electrocardiograph apparatus, and right and left punctures made in the usual way while the tracings were being taken. No significant effects were noted, one or two extra systoles occasionally appearing.[2] Pneumo-

[2] My thanks are due to Dr. E. P. Carter for taking these electrocardiograms.

thorax and hemopericardium have to be considered as possible sources of error. We have never noticed any ill effects in our dogs even where heart punctures have been made repeatedly on the same day. The best evidence that these do not occur or at least to the extent to influence the circulation is seen in the figures obtained for the minute volumes. The constancy of the minute volume when two determinations are made on the same day in any of the animals, the close agreement of the figures over long periods of time for the two male dogs even where a long interval elapses between experiments, and the fact that the output in the three females is as likely to rise as to fall on the second day of a new period of observations, would seem to preclude any significant effects on the heart.

Variations in minute volume. The data on the circulatory minute volume of the five animals used are given in tables 1, 2, 3, 4 and 5. In

TABLE 2

"Hound" male, weight on February 21, 13.9 kilos; on March 7, 14.1 kilos; on June 25, 14.9 kilos

DATE	MINUTE VOLUME	PULSE PER MINUTE	OXYGEN USED PER MINUTE	RESPIRATION LITRES PER MINUTE	ARTERIAL OXYGEN	ARTERIAL VENOUS DIFFERENCE
1924	*litres*		*cc.*		*volume per cent*	*volume per cent*
February 21.....	1.86	80	92	2.00	17.39	4.95
February 26.....	1.94	63	101	2.24	17.37	5.20
February 26.....	1.91	77	109	1.76	17.34	5.70
March 7.....	1.75	76	98	2.15	17.94	5.62
March 7.....	1.58	81	85	2.06	17.94	5.40
March 10.....	1.91	74	101	1.69	17.04	5.29
April 4.....	1.35	66	65	1.74	17.54	4.81
June 30.....	1.80	87	74	1.95	17.47	4.13
July 3.....	1.72	85	76	1.78	17.69	4.43

the tables are included data on the pulse rate, oxygen consumption, respiratory minute volume (corrected to S. T. P.), the arterial oxygen, and the difference between the arterial and mixed venous blood oxygen. An examination of the first two tables indicates that the minute volume of these dogs is fairly constant throughout the period of observation. The differences from the mean value do not exceed 10 per cent. On the other hand, the figures for the three other animals show quite wide variations, frequently 40 per cent or more from the mean value. The first two animals were males, the others females, one being studied during the course of a pregnancy. This suggested that there may be a cyclic variation in the minute volume of the female associated with oestrous cycle changes and if the figures are plotted against time some kind of a cycle is shown. The minute volumes are not, however, determined at frequent enough intervals to determine the course of the cycle with certainty. One animal (table 3)

had the highest minute volume during heat, and the pregnant dog (table 5) had a much higher output on the basis of its surface area than any of the others. It is of interest in this connection to note that Collett and

TABLE 3

"Liz," female, weight on February 7, 1924, 19.5 kilos; on March 1, 17.8 kilos; on June 18, 21.8 kilos; March 18, 1925, 22.2 kilos. During last two weeks of February, 1924, animal had a mild attack of distemper, possibly some pneumonia, completely well by middle of March. Dog was in "heat" about May 10 to May 19, 1925. On May 19, animal was found dead in cage at 8:30 a.m. In good condition 12:30 p.m. day before. Autopsy and sections made but cause of death not discovered.

DATE	MINUTE VOLUME	PULSE PER MINUTE	OXYGEN USED PER MINUTE	RESPIRATION LITRES PER MINUTE	ARTERIAL OXYGEN	ARTERIAL VENOUS DIFFERENCE
1924	*litres*		*cc.*		*volume per cent*	*volume per cent*
February 7	1.88	68	132	5.34	18.94	7.05
February 8	1.94	66	135	4.65	17.04	6.95
March 1	1.64	60	112	4.57	16.24	6.85
March 25	1.54	63	94	3.55	15.97	6.11
March 27	1.70	76	102	5.11	15.39	6.07
June 16	1.54	84	157	7.50	19.80	10.19
June 17	1.58	74	117	5.41	18.90	7.41
June 18	2.10	150	113	5.37	18.60	5.51
June 18	1.94	153	115	5.57	18.61	5.93
June 20	2.10	77	124	7.32	18.67	6.04
July 1	2.93	83	121	4.31	19.59	4.15
July 2	2.81	81	117	3.68	19.25	4.16
1925						
March 18	1.78	80	102	4.55	19.02	5.72
March 19	2.57	82	128	9.11	19.83	4.97
March 21	2.37	80	99	5.51	20.30	4.17
March 24	2.12	76	122	4.07	19.00	5.75
March 27	2.17	90	122	4.66	19.69	5.62
March 28	1.88	77	116	4.01	18.22	6.17
April 3	1.87	106	145	6.87	23.71	7.74
April 20	2.13	84	111	5.08	19.42	5.22
April 28	3.33	79	147	6.18	17.39	4.41
April 29	2.67	84	117	5.69	17.91	4.39
April 29	2.70	84	152	7.04	17.92	5.63
May 2	2.53	75	111	5.77	17.80	4.39
May 4	2.38	79	120	4.36	18.45	5.05
May 8	2.77	86	164	7.15	17.96	5.91
May 15	3.67	86	173	6.41	17.30	4.71
May 15	3.57	86	184	9.32	17.20	5.15
May 18	3.78	80	153	6.13	17.54	4.05

Liljestrand (1924) have found in one individual with the nitrous oxide method two maxima and two minima in the curve of the minute volume during a menstrual cycle. Sufficient observations have not been accu-

mulated, however, to be sure that the variations in minute volume encountered are truly related to the oestrous cycle.

The only data available to compare with ours are those obtained on normal goats. Barcroft, Boycott, Dunn and Peters (1919) report 43 experiments on 21 goats. All except two were females. For only 6 animals are more than two determinations available. On each of these from 3 to 5 estimations in all were done on three days over a period in no case longer than one week. It happens that the two males fall into this group. We have calculated from the figures that the values for the males do not differ from the mean by more than about 10 per cent, while the

TABLE 4

"Misch," female, weight on March 15, 1924, 12.3 kilos; November 29, 12.2 kilos; February 19, 1925, 11.1 kilos. On March 5, after an experiment injecting morphine and breathing carbon dioxide, animal seemed quite sick, but was much better in a couple of hours. Found dead about 1 hour later. Autopsy did not disclose cause of death.

DATE	MINUTE VOLUME	PULSE PER MINUTE	OXYGEN USED PER MINUTE	RESPIRATION LITRES PER MINUTE	ARTERIAL OXYGEN	ARTERIAL VENOUS DIFFERENCE
1924	*litres*		*cc.*		*volume per cent*	*volume per cent*
March 15	2.06	115	96	3.77	18.74	4.65
March 17	2.18	109	106	3.54	20.56	4.87
November 29	1.73	75	68	2.42	21.24	3.95
December 13	1.32	123	68	3.23	23.30	5.18
1925						
January 8	1.36	70	66	2.30	22.75	4.85
January 17	1.46	72	68	2.54	21.99	4.64
January 22	1.20	75	69	2.39	21.76	5.78
January 29	1.55	67	65	2.61	19.70	4.18
February 7	1.66	72	68	3.08	20.97	4.11
February 12	2.02	76	75	2.78	20.15	3.71
February 19	1.40	86	63	2.51	22.05	4.51
February 24	1.51	90	76	3.03	19.24	5.04
February 24	1.58	102	91	3.26	20.98	5.74
March 5	1.63	78	67	2.64	17.74	4.10

values for the females show maximum differences of 16 to 26 per cent from the mean. Although the determinations are few, the results are suggestive in view of our findings.

In spite of the large variations in minute volume which occur in some of the animals, our experience has been that changes do not occur in a few hours. Whenever, the determinations have been repeated on the same day, the values for any of the animals have not differed by more than about 10 per cent, which is the limit of accuracy of the method. This is a very important finding as it allows one to use an animal of either sex for a short experiment. If the normal determination is made, some experi-

mental procedure or drug then used, and another determination made on the same day, any change in the minute volume is definitely significant.

The effect of changes in pulse rate. Changes in pulse rate that have occurred do not seem to bear any relation to the changes in minute volume in four of the dogs. In the other, the pregnant animal, changes in pulse rate are accompanied by almost proportionate changes in minute volume. This means that the systolic output varies inversely as the pulse rate in the case of the two dogs with a constancy of the minute volume.[3] Quite a different relation is evident for Blackie, the pregnant female. Here an increase in pulse rate is accompanied by an almost proportionate increase

TABLE 5

"Blackie," female, weight at commencement of experiments 15.9 and at end 19.2 kilos. When first determination was made, animal was in "heat" and had just become pregnant. A litter of nine pups was born on July 15

DATE	MINUTE VOLUME	PULSE PER MINUTE	OXYGEN USED PER MINUTE	RESPIRATION LITRES PER MINUTE	ARTERIAL OXYGEN	ARTERIAL VENOUS DIFFERENCE
1925	*litres*		*cc.*		*volume per cent*	*volume per cent*
May 22	2.85	84	116	4.16	18.29	4.07
May 25	2.57	86	99	3.31	18.26	3.85
May 28	2.65	78	115	4.14	18.58	4.34
May 29	2.66	73	112	4.53	17.67	4.21
June 1	1.90	64	101	3.73	17.70	5.32
June 3	2.44	66	104	5.24	16.45	4.27
June 15	2.60	68	98	5.54	13.56	3.79
June 16	2.41	76	108	5.46	13.22	4.47
June 18	2.59	80	125	6.28	13.80	4.81
June 24	3.13	93	122	7.30	13.10	3.90
June 25	2.90	100	118	7.45	12.48	4.07
July 3	3.25	112	170	9.50	12.89	5.23
July 6	3.24	112	123	8.65	11.85	3.80
Aug. 5	3.79	118	159	5.40	15.00	4.20
Aug. 6	3.84	118	153	6.60	14.30	3.98

in minute volume or, in other words, the systolic output appears to be constant.

The explanation of the above findings probably lies in the nature of the venous filling of the heart. Krogh in 1912 pointed out that in an inadequately filled heart, one in which the venous return and pressure was not sufficient to fill it to its maximum capacity during the period of diastole, an increase in pulse rate would be accompanied by only very slight changes in total minute output. On the other hand, in an adequately filled heart,

[3] A number of experiments have been made on the effect of markedly increasing the pulse rate by the injection of atropine. In general, no effect on minute volume has been observed. These experiments will be reported in detail in another paper.

increase of pulse rate would cause an increase in minute volume. This would mean that in the normal dog we are dealing with an inadequately filled heart, while in the pregnant animal the heart is adequately filled. Experiments on operated, anesthetized animals have led many to regard the normal heart as an adequately filled heart. It seems, however, impossible to explain the lack of relation of minute volume to pulse rate on this idea in the normal dog. Of course, if the pulse becomes sufficiently slow to lengthen the period of diastole until the heart is completely filled any further slowing will be accompanied by a decrease in minute volume. Pulse rate changes, however, from 60 upwards are without influence on the total minute output within the limits of error of our method. The investigation of Meek (1924) on the effect of changes in pulse rate on the diastolic size of the heart brings up an important consideration in view of our results. Meek found in morphinized and in unanesthetized animals that changes in pulse rate from about 55 to 110 per minute are accompanied by only slight changes in the diastolic x-ray shadow of the heart. The work reported here shows that pulse rate changes from 60 to 110 are not accompanied by changes in minute output, and, therefore, there must be inversely proportionate changes in systolic output. This can only mean that these changes in output per beat are due mainly to variations in the systolic size of the heart and not the diastolic.

The effect of changes in oxygen consumption. Several investigators (Means and Newburgh, 1915; Boothby, 1915; Lindhard, 1915) concluded as the result of experiments on man that the minute volume of the heart varies directly with the oxygen consumption of the body, the utilization, therefore, being constant. The figures given in our table are quite sufficient to show that such a view does not hold for the normal dog. Changes of 30 per cent or more may be seen in the oxygen used with no change in the minute volume or even a change in the opposite direction. In some animals there appears to be some correlation between the oxygen consumption and the minute volume, in others absolutely none. That marked changes in the utilization of oxygen can occur is seen from an examination of the columns in the tables giving the differences in oxygen percentages between arterial and mixed venous blood.

Effect of changes in respiration. Marked changes (100 per cent or more) in the ventilation of the lungs are not accompanied by changes in the minute volume. It can be seen from an examination of the tables that there is no apparent correlation between ventilation and minute volumes.

Effect of changes in arterial oxygen. Since the arterial blood in all of our experiments has been bright red and therefore nearly saturated with oxygen, the values for the arterial oxygen represent fairly accurately the hemoglobin content of the blood. In Brownie (table 1) we see the most marked changes in arterial oxygen with no corresponding changes in

minute volume. The maximum arterial oxygen value is 21.45 vol. per cent with a minute volume of 2.51 litres, and the minimum is 12.72 vol. per cent with a minute volume of 2.53 litres. This means that the utilization of oxygen is not at all constant during rest. Where both the arterial oxygen and oxygen consumption have markedly changed we get widely different utilizations. Thus, on February 4, 1924, the utilization of arterial oxygen is about 44 per cent, while on October 7, 1925, it is only about 17 per cent. In Hound and Misch, the values for arterial oxygen are fairly constant, while in Liz (table 3) marked variations occur, but no relation in these is seen to changes in minute volume. In the pregnant dog (table 5) some correlation between arterial oxygen and minute volume may exist. However, the data on Brownie where the widest variations occur indicate clearly that moderate anemia is not compensated for by an increased circulation.

The effect of exposure to cold. The fact that no relation is apparent between pulse rate and minute volume means that the systolic output is quite variable. The question of the constancy or variability of the systolic output has been discussed at great length for many years. The most important point of issue has been the behavior of the systolic output during exercise. Although we have not made any experiments on our animals during active muscular exercise, the heart output has been studied during a condition corresponding to mild exercise. The animal was given a cold bath and then exposed to a fairly low external temperature. Marked shivering was produced which lasted for a sufficient time to allow a determination to be made. A marked increase in systolic output was found. The following table gives the result of two experiments.

TABLE 6

Effect of exposure to cold on minute volume

	OXYGEN PER MINUTE	PULSE	MINUTE VOLUME	SYSTOLIC OUTPUT
	Brownie			
	cc.		*litres*	*cc.*
Normal	104	83	2.71	32.7
Cold	232	120	5.31	44.3
	Misch			
Normal	66	70	1.36	19.4
Cold	236	97	3.66	37.7

Somewhat similar results have been reported for man by Barcroft and Marshall (1924). They found that exposure to cold just about sufficient to produce shivering caused an increase in minute volume and a decrease in pulse rate which would mean a marked increase in systolic output.

Variation in minute volume in different animals. On account of the marked variations in the minute volume in three of the dogs, it is not easy to compare the values for different animals. The mean value for the minute volume of the different dogs has been compared in the following table. Surface area seemed to be a more satisfactory method of comparison than weight. The minute volume per square meter is very constant for three of the animals, is slightly greater for another, and markedly greater for the pregnant dog, Blackie. The weights given are the average weights during the course of the experiments with the exception of that for Brownie. This dog was so evidently ill-nourished and underweight at the beginning of the observations, that the weights of the earlier weeks

TABLE 7

Minute volume per square meter body surface

DOG	WEIGHT	SURFACE AREA	MINUTE VOLUME	MINUTE VOLUME PER SQUARE METER
	kilos		*litres*	
Brownie ♂	18.0	0.77	2.61	3.39
Hound ♂	14.5	0.67	1.76	2.64
Liz ♀	20.0	0.83	2.19	2.66
Misch ♀	12.0	0.59	1.62	2.76
Blackie ♀	16.0	0.71	2.86	4.02

TABLE 8

DOG	MINUTE VOLUME			CORRELATION OF MINUTE VOLUME WITH			
	Mean	σ	C. of V.	Pulse	Oxygen per minute	Respiration	Arterial Oxygen
Hound ♂	1.758±0.040	0.180±0.029	10.2±1.6	0.098±0.223	0.779±0.088	0.200±0.216	−0.527±0.162
Brownie ♂	2.609±0.023	0.162±0.017	6.2±0.6	−0.094±0.143	−0.276±0.133	−0.477±0.111	−0.284±0.132
Liz ♀	2.191±0.062	0.471±0.044	21.5±2.1	0.032±0.132	0.321±0.119	0.199±0.127	0.067±0.132
Misch ♀	1.619±0.051	0.281±0.036	17.4±2.3	0.285±0.166	0.719±0.087	0.060±0.160	−0.531±0.129
Blackie ♀	2.855±0.088	0.504±0.062	17.6±2.2	0.919±0.027	0.822±0.056	0.535±0.124	−0.412±0.145

have not been used for an average. The surface area was calculated from Meeh's formula using Rubner's constant for the dog: Surface area in sq. m. $= 0.112\sqrt[3]{\text{wt. in kilos}^2}$.

Statistical study of the results. Through the kindness of Dr. Raymond Pearl the results given in the tables 1 to 5 have been subjected to partial biometrical analysis. The following table gives a summary. The standard deviation and coefficient of variation are given in columns 3 and 4. They show the greater variability of the three females than the two males, which has already been discussed. The correlation coefficients of the minute volume with pulse rate, oxygen per minute, ventilation and arterial oxygen are given in columns 5, 6, 7 and 8. They bear out in general what has already been said of these factors.

SUMMARY

The minute volume of the circulation has been determined on a series of five normal unanesthetized dogs by the direct application of the Fick principle. These determinations have been repeated at various intervals on the animals for periods of time extending in one case over two years.

Two animals have shown a very constant figure for the minute volume, while the three others have shown much more variation. The first two animals were males, the last three females, one pregnant.

In the normal dog changes in pulse rate which occur spontaneously are without effect on the minute volume. The systolic output appears to vary inversely as the pulse rate.

Either the oxygen consumption or the respiration may change considerably without affecting the minute volume.

Shivering caused by exposure to cold causes a marked increase in the output per beat.

BIBLIOGRAPHY

Barcroft, Boycott, Dunn and Peters. 1919. Quart. Journ. Med., xiii, 34.
Barcroft and Marshall. 1923. Journ. Physiol., lviii, 145.
Bock and Buchholtz. 1920. Arch. f. Exper. Path. u. Pharm., lxxxviii, 192.
Bohr and Henriques. 1897. Arch. de Physiol. norm. et path., 5th Ser., ix, 459.
Boothby. 1915. This Journal, xxxvii, 383.
Burwell and Robinson. 1924. Journ. Clin. Inves., i, 87.
Collett and Liljestrand. 1924. Skand Arch. f. Physiol., xlv, 17.
Doi. 1921. Journ. Physiol., lv, 43.
Douglas and Haldane. 1922. Journ. Physiol., lvi, 69.
Elving and v. Wendt. 1907. Skand. Arch. f. Physiol., xix, 96.
Eyster and Meek. 1920. Amer. Journ. Roentgenol., vii, 471.
Fick. 1870. Sitzungsb. der phys.-med. Gesellsch. zu Wurzburg, 16.
Field, Bock, Gildea and Lathrop. 1924. Journ. Clin. Inves., i, 65.
Grehant and Quinquaud. 1886. C. R. Soc. Biol., 159.
Harrison, Dock and Holman. 1924. Heart, xi, 337.
Henderson. 1906. This Journal, xvi, 325.
1909. This Journal, xxiii, 345.
1923. Physiol. Rev., iii, 165.
Henderson and Haggard. 1925. This Journal, lxxiii, 193.
Henderson and Prince. 1917. Journ. Biol. Chem., xxxii, 325.
Hering. 1829. Zeitschr. f. Physiol., iii, 85.
Hill. 1913. Journ. Physiol., xlvi (Proc. xxvi).
Huggett. 1924. Journ. Physiol., lix, 373.
Johansson and Tigerstedt. 1889. Skand. Arch. f. Physiol., i, 331; 1891. Skand Arch. f. Physiol., ii, 409.
Kitchen. 1924. This Journal, lxvii, 493.
Krogh and Lindhard. 1912. Skand. Arch. f. Physiol., xxvii, 100.
Krogh. 1912. Skand. Arch. f. Physiol., xxvii, 126.
Lindhard. 1915. Pflüger's Arch., clxi, 233.
Loewy and Schrötter. 1905. Zeitschr. f. Exper. Path. u. Therap., i, 197.

MEANS AND NEWBURGH. 1915. Journ. Pharm. Exper. Therap., vii, 449.
MEEK AND EYSTER. 1922. This Journal, lxi, 186.
MEEK. 1924. This Journal, lxx, 385.
MURLIN AND GREER. 1914. This Journal, xxxiii, 253.
ODAIRA. 1925. Tohoku Journ. Exper. Med., vi, 325.
PLESCH. 1909. Zeitschr. f. Exper. Path. u. Therap., vi, 380.
ROY AND ADAMI. 1892. Phil. Trans., clxxxiii, B, 202.
STARLING AND EVANS. 1913. Journ. Physiol., xlvi, 413.
STEWART. 1894. Journ. Physiol., xv, 1.
1921. This Journal, lvii, 27; lviii, 20, 278.
TIGERSTEDT, C. 1909. Skand. Arch. f. Physiol., xxii, 115.
1922. Acta Med. Scand., lvi, 510.
TIGERSTEDT, R. 1891. Skand. Arch. f. Physiol., iii, 145.
1905. Ergebn. der Physiol., iv, 481.
1921. Physiologie des Kreislaufes, i, 179.
UYENO. 1922. Journ. Physiol., lvii, 14.
VAN SLYKE AND NEILL. 1924. Journ. Biol. Chem., lxi, 523.
VAN SLYKE AND STADIE. 1921. Journ. Biol. Chem., xlix, 1.
ZUNTZ AND HAGEMANN. 1898. Landwirtsch. Jahrbücher, Suppl. iii, xxvii, 371.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE PANCREAS

I. A Method for the Subcutaneous Auto-Transplantation of the Tail of the Pancreas[1]

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We have briefly described a method (Ivy and Farrell, 1925) for the auto-transplantation of a small pouch of the stomach subcutaneously. We have used the principles of this method in transplanting the tail of the pancreas subcutaneously.

On reviewing the literature we found that our method for auto-transplantation of the pancreas is essentially the same as that used first by Minkowski (1892, 1893), and later by Hedon (1892, 1898) and Thirloix (1892, 1892a, 1895). Minkowski performed only a pseudo-transplantation in six dogs. He did not completely section the intra-abdominal pedicle in any of his experiments. In one experiment he sectioned the vein, but not the artery. One experiment was a complete failure, and in another marked atrophy of the transplant occurred. The pseudo-transplant functioned in three experiments to the extent that glycosuria did not occur on the complete removal of the remainder of the pancreas. He observed a flow of pancreatic juice from five of the transplants. Hedon sectioned the intra-abdominal pedicle and therefore performed an auto-transplant. He reported one successful experiment, and several years later (1898) two additional experiments out of a rather large series in which the pedicle was tied without diabetes resulting. Hedon observed that the transplants secreted externally and reported that such transplants might serve as permanent pancreatic fistula preparations. Thirloix has reported four successful auto-transplants of the tail of the pancreas; but because of failures, he attempted to produce atrophy of the pancreas by injecting the ducts with oil and lamp-black before transplantation was undertaken. These experiments were not very successful. Thirloix also observed that the transplants secreted active pancreatic juice, in one case as much as fifteen cubic centimeters per day. None of these investigators mentioned any relationship between the ingestion of a meal and the rate of flow of juice.

[1] Preliminary report and demonstration given at the meetings of the Amer. Physiol. Soc., Cleveland, December, 1925.

As far as we have been able to ascertain, no one has realized the possibilities of this method beyond its direct relation to the proof of an internal secretion of the pancreas, which it does not prove unequivocally.

Since this method can be used for the solution of numerous problems bearing on the external and internal secretion of the pancreas, we desire

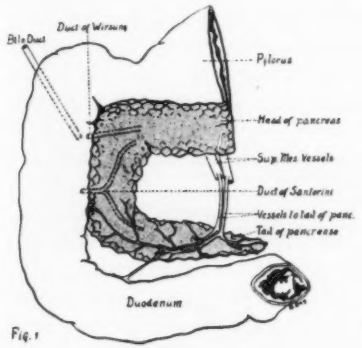

Fig. 1

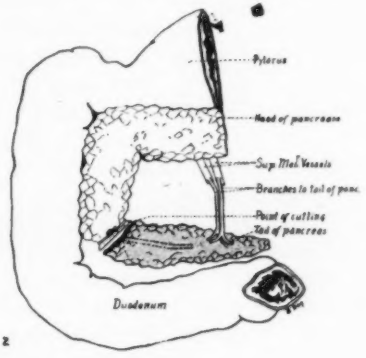

Fig. 2

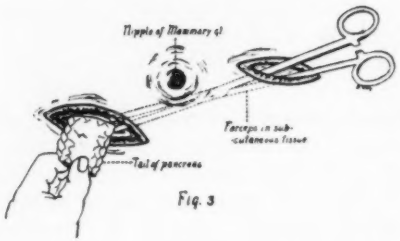

Fig. 3

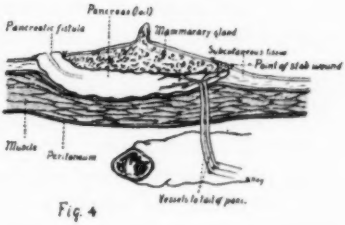

Fig. 4

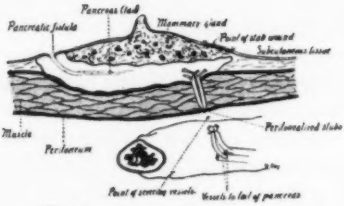

Fig. 5

to describe in detail the technique we use, especially since this technique has proven to be very successful.

METHOD. We use female dogs that have recently whelped or weaned pups. This insures a vascular bed. Male dogs can be used but success is not so certain and greater atrophy of the transplant occurs.

FIRST STAGE. *First step.* A right rectus incision is made one quarter

of an inch from the midline. The duodenum and pancreas are delivered through the incision, this being made possible by sectioning a portion of the gastro-hepatic ligament. The usual anatomical picture shown in figure 1 is presented. The mesentery on each side of the tail of the pancreas is severed, small blood vessels being ligated when necessary. Usually it is necessary to ligate only one artery and vein which pass to and from the duodenum. The smallest possible number of ligations is used, as it is desirable to reduce the amount of "foreign-body reaction" in the region of the transplant. Care must be taken in handling the pancreas during this procedure because of possible trauma. It is not necessary to touch the pancreas up to this time. The pancreas and duodenum are now returned to the abdominal cavity for protection while the subcutaneous bed is being prepared.

Second step. A stab-wound incision about 2 cm. long is made entirely through the abdominal wall about 5 cm. cephalad to the nipple. It should be sufficiently large to permit free passage of the tail of the pancreas. Care is taken in making the stab-wound not to incise the larger blood vessels. About 5 cm. caudad to the nipple a skin incision is made about 2 cm. long. Care is exercised not to incise the larger blood vessels. With a hemostat the areolar tissue just beneath the mammary gland is tunnelled and spread apart between the skin and stab-wound incisions and about the stab-wound, sufficiently to prepare an ample but snug bed for the transplant. (See fig. 3.)

Third step. The duodenum and pancreas are delivered and a double silk ligature is passed around the pancreas at the inferior pancreaticoduodenal angle including as much of the body of the pancreas as possible, and tied. The tail of the pancreas is freed from the body of the pancreas by sectioning with the scissors just distal to the ligature. (See fig. 2.) A piece of gauze is placed over the raw cut stump to control hemorrhage. No ligatures are necessary. Free bleeding soon ceases, but oozing of blood may occur and is permitted to continue a number of hours. This is the first time that it is necessary to touch the tail of the pancreas.

Fourth step. A hemostat is passed through the stab-wound into the abdomen; the raw stump of the tail of the pancreas is grasped and drawn through the stab-wound until the tail of the pancreas is outside the abdomen, the pedicle, consisting of the blood vessels and nerve fibers, pass-

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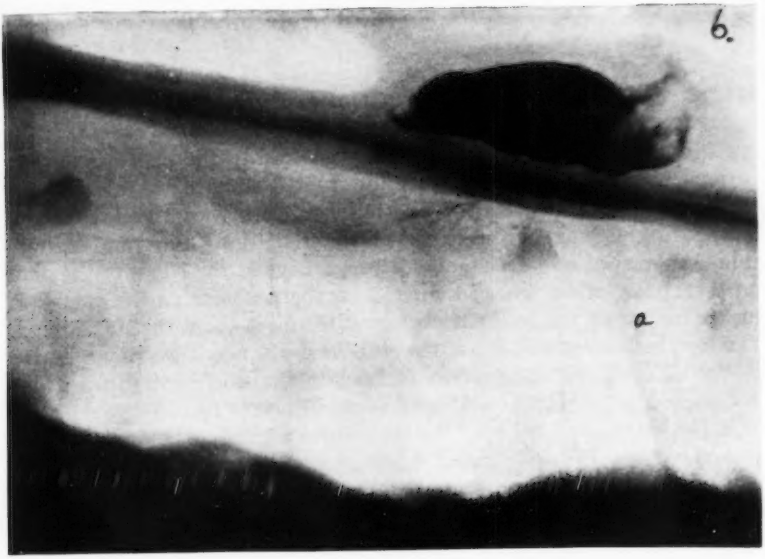
Fig. 6. Pneumoperitoneum of a dog with a gastric pouch transplant. Note adhesion at *a*.

Fig. 7. Pneumoperitoneum of the same dog shown in figure 1 after the adhesion was removed.

Fig. 8. Pneumoperitoneum of a dog with a pancreatic auto-transplant. The needles show the location of the transplant. There are no connections between the transplant and the underlying viscera.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a


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ing through the stab-wound incision. The tail of the pancreas is then covered with gauze. Two or three interrupted stitches are taken in the peritoneal edges of the stab-wound incision, closing the incision snugly, but not firmly. A hemostat is passed from the skin incision, and the raw stump is grasped and drawn about 3 mm. beyond the edges of the skin incision. Any of the tail of the pancreas that remains on the outside is tucked under the skin in the pocket prepared for it about the stab-wound. The skin incision at the site of the stab-wound is now closed by a buried subcuticular stitch. The stump is fixed to the skin incision with four interrupted sutures. The abdomen is closed. (See fig. 4.)

Sterile gauze is placed over the stump and a bandage is applied. The animal is dressed daily. In the course of two weeks the skin grows over the stump of the pancreas up to the duct, but not over it. In only two animals out of twelve has there been any tendency for the duct to be closed by skin.

SECOND STAGE. Three or four weeks later. A small abdominal incision not more than 4 cm. long is made about an inch to one side of the pancreatic "transplant" at the point at which it is known that the pedicle can be easily reached. Through this incision the pedicle—now a cord about the size of a match stick—is doubly ligated and cut between, the stump of the pedicle attached to the peritoneum being peritonialized to prevent adhesions. The abdomen is closed. (See fig. 5.)

RESULTS. *Proof of the transplant.* We have proved the absence of adhesions or connections with underlying viscera—by either exploratory operation or pneumo-peritoneum. (See figs. 6 to 8.[2])

Percentage of success. We have had no failures. We have done twelve of these operations up to the present time. The marked edema described by Minkowski, Hedon and Thirloix has occurred only occasionally.

Survival of the transplant. We now have animals in which the transplant was made over a year ago.

Secretion of the transplant. The transplant produces both an external and an internal secretion. It is about 50 per cent atrophied as calculated from the amount of external secretion that the amount of pancreas transplanted should secrete. In some animals the amount of atrophy is less than this because it must be kept in mind that we are getting only the secretion due to a humoral mechanism. More details will be given in later communications.

DISCUSSION. We attribute our high percentage of success to four factors: 1, the selection of a female with developed and vascular mammary glands; 2, care in handling the transplant; 3, the use of very little suture material; 4, the exposure of the entire stump to the exterior which insures

[2] The radiograms and pneumo peritoneums were made for us by Dr. B. F. Orndoff, North Chicago Hospital.

better drainage of the juice from the transplant and renders closure of the duct less likely.

This method, we believe, can be used not only for the solution of problems related to the external secretion of the pancreas, but also for the solution of problems related to the etiology of diabetes mellitus.

SUMMARY

A very successful method is described for the subcutaneous transplantation of the tail of the pancreas in the dog. The transplant produces both the external and the internal secretion of the pancreas.

BIBLIOGRAPHY

HEDON. 1892. Compt. Rend. Soc. de Biol., xliv, 307, 678, 763.
1898. Travaux de physiologie, Paris, cited by HEDON in Rev. de Med., 1910, xxx, 622.
IVY AND FARRELL. 1925. This Journal, lxxiv, 639.
LOMBROSO. 1910. Ergebn. d. Physiol., ix, 11.
MINKOWSKI. 1892. Berl. Klin. Wchnschr., xxix, 90.
1893. Arch. f. exper. Path. u. Pharm., xxxi, 118.
THIRLOIX. 1892. Arch. de Physiol., xxiv, series 5, iv, 617.
1892a. Compt. rend. Soc. de Biol., xliv, 966.
1895. Compt. rend. Soc. de Biol., xlvii, 256.

THE EFFECT OF AGE ON THE HEMOGLOBIN OF THE RAT

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In a previous study (1) on the effect of iron in anemia a great many hemoglobin determinations were made on normal rats. It was observed that the hemoglobin concentration seemed to vary with the age of the rats and it was suggested by one of the authors (C. S. W.) that the hemoglobin of rats varied in the same manner as he had shown it to vary in the human being (2).

A re-grouping was made of 730 hemoglobin determinations according to the age of the rats and a normal hemoglobin curve obtained.

All the determinations used in this present study were on normal rats which were raised in this laboratory on the standard casein diet. They grew and reproduced normally.

The hemoglobin was determined spectrophotometrically, the details of the method being given in previous papers (1), (2).

RESULTS. The results of the 730 determinations are given in table 1. The determinations were grouped into classes which represent twenty-day intervals according to the age of the rat at the time of making the observation. The mean, or average value of each group was then determined and the probable error of this mean calculated. The average of all the 730 observations was also obtained with its probable error.

The probable error is a measure of the reliability of the results and also gives the range of value in which 50 per cent of all the observations will be found.

The measurement in the rat of hemoglobin concentration in the previous paper was given in terms of extinction coefficients—a factor which varies directly with hemoglobin concentration and which can be converted into grams of hemoglobin per 100 cc. by multiplying it by the absorption coefficient for the blood of rats and, of course, by the dilution. The absorption coefficient for rats' blood was determined by Welker and Williamson (3) and found to be 0.001130. The dilution factor for the pipette was 110.71.

DISCUSSION. It should be noted that the hemoglobin concentration steadily falls during the first fifty days of the rat's life and then gradually rises until about the 150th day when a maximum is reached. Thereafter the value again falls to a level which it maintains.

Our results do not agree exactly with those of other writers although the same general relationship holds true. Donaldson (4) quoting Chisolm (5) and also Abderhalden (6) reports that the hemoglobin percentage is high at the birth of rats and then gradually falls until about the 30th day when a sudden regeneration takes place and the animals attain a normal value which they maintain throughout life. Chisolm says, however, that his figures for rats after the 16th day must be received with caution because "the rats did not seem to grow as they should have done although they appeared healthy enough." These early writers did not use as large a number of animals as were studied in this experiment and their methods for hemoglobin determination and care as to the diet of laboratory ani-

TABLE 1

Table showing grams of hemoglobin per 100 cc. of blood at each age period, and the number of the determinations for each period

AGE RANGE	MID POINT AGE	NUMBER OF DETERMINATIONS	MEAN VALUE, GRAMS HEMOGLOBIN PER 100 CC. BLOOD	EXTINCTION COEFFICIENT
days	*days*			
1–19	10	25	12.94 ± 0.23	1.035 ± 0.018
20–39	30	77	12.62 ± 0.20	1.009 ± 0.016
40–59	50	205	12.44 ± 0.16	0.994 ± 0.013
60–79	70	101	13.40 ± 0.17	1.072 ± 0.014
80–99	90	93	13.54 ± 0.21	1.082 ± 0.017
100–119	110	38	14.04 ± 0.30	1.175 ± 0.024
120–139	130	3	14.91 ± 0.46	1.192 ± 0.037
140–159	150	14	15.51 ± 0.25	1.240 ± 0.020
160–179	170	52	13.90 ± 0.25	1.111 ± 0.020
180–199	190	76	13.70 ± 0.20	1.095 ± 0.016
230–250	240	46	13.80 ± 0.24	1.103 ± 0.019

Total number of determinations, 730.
Average of 730 determinations, 13.77 ± 0.24.

mals are open to question. The failure of Chisolm's rats to grow might well indicate a deficiency disease.

The explanation of this variation in the hemoglobin curve has been given by Bunge (7) who has shown with dogs that the ash of a new-born puppy contains six times as much iron as does the bitch's milk; and also that the iron in the liver is very much higher immediately after birth than in adult animals. Abderhalden (8) has shown the same results for rabbits and rats. Thus the amount of iron in milk being small the animals gradually become anemic during the suckling period which lasts up until about the 21st to the 25th day. After the rats begin to eat and take in iron from the outside this anemia gradually disappears. It will be noted that we give no figures for the new-born rat. This is because of the difficulty with the method used by us of obtaining a sufficient quantity of blood for satis-

factory determinations. Judging from the slope of the curve from the 10th to the 30th day, it is highly probable that the figures at birth would be somewhat higher.

CONCLUSION

The hemoglobin concentration of the rat's blood varies with the age of the animal, in somewhat the same way as has been already established for human blood

BIBLIOGRAPHY

(1) WILLIAMSON, C. S. AND H. N. ETS. Arch. Int. Med., 1925, xxxvi, 333.
(2) WILLIAMSON, C. S. Arch. Int. Med., 1916, xviii, 505.
(3) WELKER AND WILLIAMSON. Journ. Biol. Chem., 1920, xli, 75.
(4) DONALDSON, H. H. The rat. Philadelphia.
(5) CHISOLM, R. A. Quart. Journ. Exper. Physiol., 1911, iv, 207.
(6) ABDERHALDEN. Zeitschr. f. Physiol. Chem., 1901–02, xxiv, 500.
(7) BUNGE. Textbook of Physiological and Pathological Chemistry. (Transl. by Wooldridge), 1890, London.
(8) ABDERHALDEN. Zeitschr. f. Physiol. Chem., 1899, xxvi, 498; Zeitschr. f. Biol., 1902, xliii, 125.

THE PHENOMENON OF TREPPE IN INTACT HUMAN SKELETAL MUSCLE

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The presence of the staircase phenomenon in excised skeletal muscle is so easily demonstrated in the laboratory that it needs no discussion here. Quite a number of successful attempts to demonstrate this phenomenon in intact skeletal muscle have been made but as far as the writers are able to find, although recognized, it has not been recorded in the case of man.

Both the production and the cause of "treppe" have been studied by numerous investigators since its recognition by Ranke (1865) and its demonstration by Bowditch (1871). It has been observed principally in the muscles of a frog and cat. The cause of "treppe," as shown by Lee (1907), is the physiological action of small amounts of the commonly recognized fatigue substances. Gruber (1923) concludes, while studying this phenomenon in cat's muscle, that it is due to increased irritability of the muscle, which is probably the result of the accumulation of small amounts of the commonly known fatigue products.

Since the knee-jerk is generally recognized as an index to neuro-muscular irritability it is used as the basis for the conclusions drawn in this paper. It seems reasonable to suppose that if the so-called fatigue products cause an increase in the irritability of the muscle, the knee-jerk would, in turn, be influenced likewise during this period of increased irritability.

Brown (1925), while studying the effect of exercise on the skeletal muscle, observed what he suspected might be "treppe." The present investigation was undertaken with the idea of determining whether or not this phenomenon could be demonstrated in man.

In order to produce conditions comparable to those which cause "treppe" in simple nerve-muscle preparations, the subjects submitted to exercise of various types. The knee-jerk was recorded by means of an apparatus described by Tuttle (1924), under each experimental condition, the average height being used as an index to the condition of the neuro-muscular mechanism at any given time.

Group A. In this experiment five subjects, trained in knee-jerk experi-

ments, skipped the rope for gradually increasing periods. First a normal-knee-jerk record was taken. Then the following routine was carried out:

¼ minute exercise with 7 minutes rest 7¼ minutes
½ minute exercise with 7 minutes rest 7½ minutes
1 minute exercise with 7 minutes rest 8 minutes
2 minutes exercise with 7 minutes rest 9 minutes
4 minutes exercise with 7 minutes rest 11 minutes
6 minutes exercise with 7 minutes rest 12 minutes
8 minutes exercise with 7 minutes rest 15 minutes

Knee-jerk and reaction time records, the results of which will appear later, were taken during the rest periods. The results are shown in table 1.

TABLE 1

Comparative height in millimeters of the knee-jerk after successive periods of rope-skipping

SUBJECT NUMBER	NORMAL KNEE-JERK	AVERAGE HEIGHT OF KNEE-JERK AFTER ROPE-SKIPPING									
		1	2	3	4	5	6	7	8	9	10
8	10.28	12.94	13.47	13.02	15.77	4.03	2.00	0.00	0.00	0.00	0.00
9	32.45	30.84	35.95	40.57	30.59	18.00	0.00	0.00	0.00	0.00	0.00
10	24.00	31.21	32.03	39.56	29.55	29.45	19.70	14.82	0.00	0.00	0.00
11	53.19	52.10	58.18	54.90	55.84	53.54	55.00	58.39	53.08	49.50	41.69
12	20.21	25.41	26.10	31.75	29.64	28.03	26.22	19.74	17.69	10.60	0.00

The data obtained from subjects 9 and 12 are shown graphically in figure 2. The records from which these data were obtained are shown in figures 5 and 6. A graphic representation of the average of all corresponding periods for this group is shown in figure 4.

Group B. Seven subjects, trained in knee-jerk experiments were used in this group. A normal knee-jerk record was taken first. Then the following routine was carried out:

1 trip down and up 54 steps followed by 5 minutes rest
2 trips down and up 54 steps followed by 5 minutes rest
3 trips down and up 54 steps followed by 5 minutes rest
4 trips down and up 54 steps followed by 5 minutes rest
5 trips down and up 54 steps followed by 5 minutes rest
6 trips down and up 54 steps followed by 5 minutes rest
7 trips down and up 54 steps followed by 5 minutes rest
8 trips down and up 54 steps followed by 5 minutes rest
9 trips down and up 54 steps followed by 5 minutes rest
10 trips down and up 54 steps followed by 5 minutes rest

Knee-jerk records were made during each rest period. The results are shown in table 2.

The data obtained from subjects 5 and 7 are shown graphically in figure 1. The records of these subjects from which the data were collected are shown in figures 7 and 8. A graphic representation of the averages of all of the corresponding periods for this group is shown in figure 3.

In order to determine whether or not the marked change in tonus as exhibited by the knee-jerk was due to exercise, control records were run on subjects 2, 8, 10, 11 and 12. The results of these controls are shown in table 3.

TABLE 2

Comparative height of the knee-jerk after successive periods of stair-climbing

SUBJECT NUMBER	NORMAL KNEE-JERK	AVERAGE HEIGHT OF KNEE-JERK AFTER STAIR-CLIMBING									
		1	2	3	4	5	6	7	8	9	10
1	26.30	29.56	24.30	18.11	22.60	13.87	9.24	5.62	5.54	3.55	0.00
2	21.93	26.00	26.61	19.68	10.77	9.72	8.45	8.25	10.17	2.11	0.00
3	32.17	30.46	28.70	28.37	24.00	22.80	20.26	16.16	0.00	0.00	0.00
4	23.37	41.45	38.31	32.41	39.53	36.73	41.36	30.16	25.45	0.00	0.00
5	36.06	44.74	43.56	41.03	36.34	38.94	34.00	31.15	34.50	0.00	0.00
6	3.27	5.67	4.76	4.42	2.38	0.94	0.00	0.00	0.00	0.00	0.00
7	13.80	21.92	26.24	27.53	24.09	36.00	14.02	12.34	8.43	7.29	5.92

TABLE 3

Comparative height of the knee-jerk after periods of rest conforming to the periods of exercise in the experimental series

SUBJECT NUMBER	NORMAL KNEE-JERK	AVERAGE HEIGHT OF THE KNEE-JERK AFTER PERIODS OF REST CORRESPONDING TO EXERCISE				
		1	2	3	4	5
10	28.1	26.3	22.7	22.4	25.3	26.8
8	18.8	19.6	23.7	23.8	23.9	
11	38.8	33.2	29.7	26.7	24.3	25.0
2	30.2	32.7	20.7	33.1	34.0	
12	27.4	25.2	24.7	25.2	25.9	26.5

The method of procedure was the same as for the experimental series, except the exercise was omitted. Figure 9 shows graphically the results obtained from this group of subjects.

Figure 9A shows graphically the average height of the knee-jerk for the control series and is comparable to figures 3 and 4.

DISCUSSION. With but one exception the records of all subjects show an increase in the extent of the knee-jerk after short periods of exercise. This exception appears among the subjects who volunteered for stair-climbing. It is reasonable to suppose that one period of stair-climbing carried the one subject over the period in which "treppe" would have appeared.

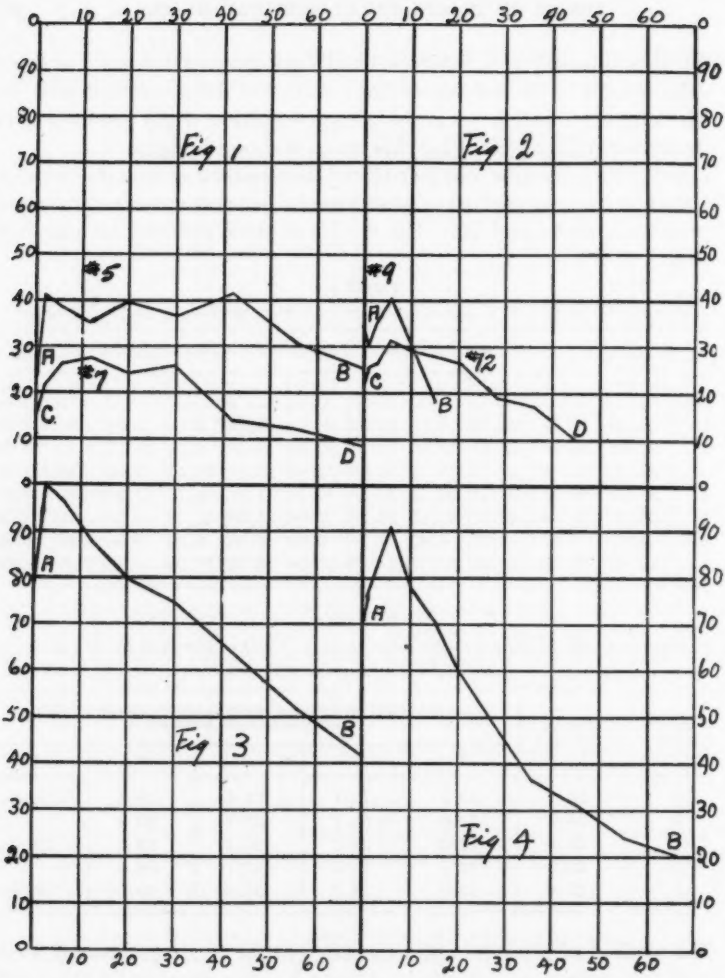

Fig. 1. Effect of stair-climbing on the height of the knee-jerk of subjects 5 and 7; 1 mm. on the abscissa equals one trip up and down 54 steps; 1 mm. on the ordinate equals 1 mm. height of the knee-jerk.

Fig. 2. Effect of rope-skipping on knee-jerk of subjects 9 and 12; 1 mm. on the abscissa equals ½ min. rope-skipping; 1 mm. on the ordinate equals 1 mm. height of knee-jerk.

Fig. 3. Average height of the knee-jerk for successive periods of stair-climbing; 1 mm. on abscissa equals one trip up and down 54 steps; 3½ mm. on the ordinate equals 1 mm. height of knee-jerk.

Fig. 4. Average of the height of the knee-jerk for successive periods of rope-skipping; 1 mm. on the abscissa equals ½ min. of rope-skipping; 2½ mm. on the ordinate equals 1 mm. height of knee-jerk.

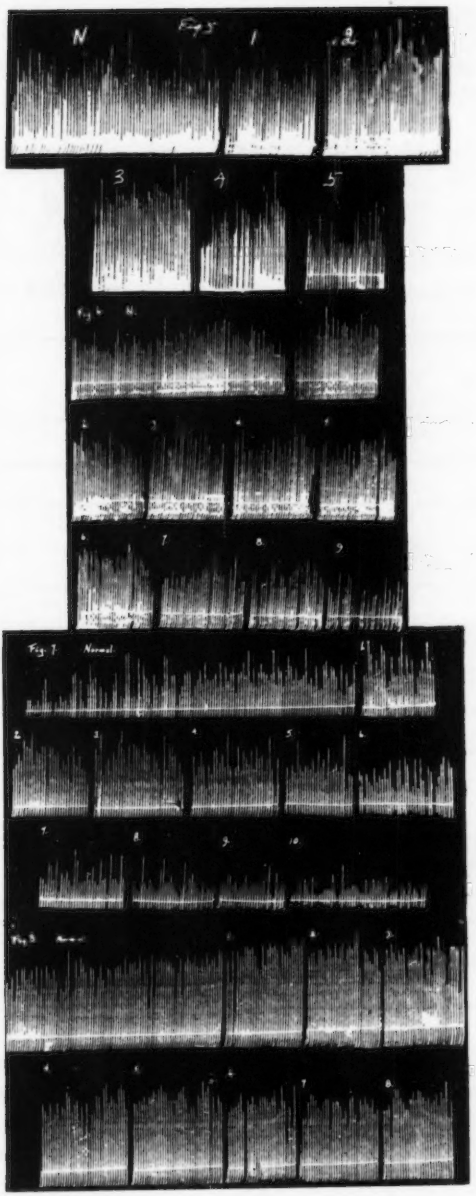

Fig. 5. Knee-jerk record of subject 9 (see table 1).
Fig. 6. Knee-jerk record of subject 12 (see table 1).
Fig. 7. Knee-jerk record of subject 7 (see table 2).
Fig. 8. Knee-jerk record of subject 5 (see table 2).

The amount of exercise necessary to bring about "treppe" varies with the individual subject. This phenomenon appeared during the first period

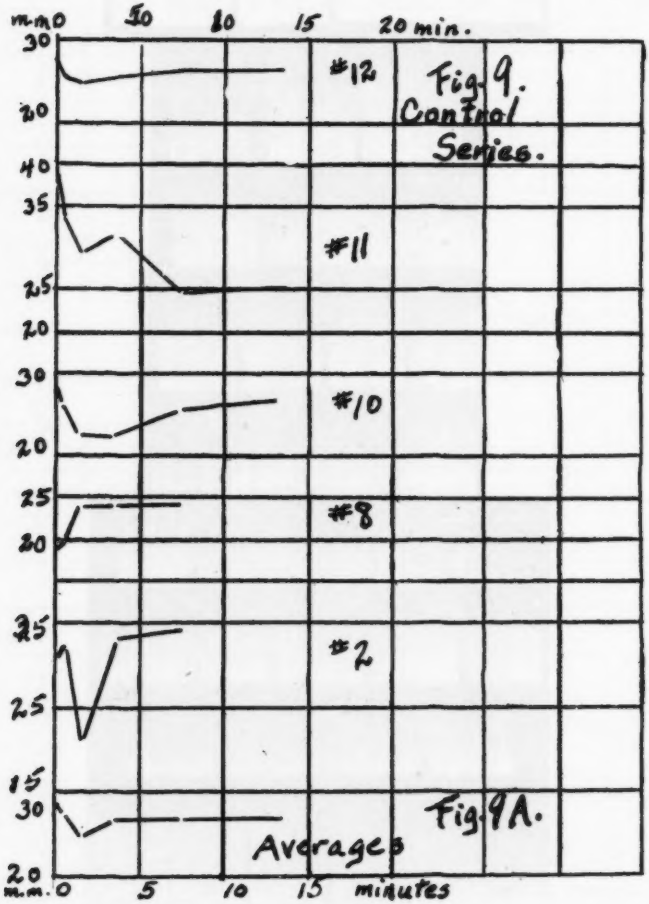

Fig. 9. Height of the knee-jerk of each subject of the control series; 1 mm. on the abscissa equals ½ minute period of rest; 1 mm. on the ordinate equals 1 mm. height of knee-jerk.

Fig. 9A. Average height of the knee-jerk of the control series. Scale same as figure 9.

of exercise in nine cases, during the second period in two cases, and not at all in one case.

Changes in the extent of the knee-jerk are evident in the records of five subjects which are comparable to changes due to contracture in records made from excised skeletal muscle. These changes are shown graphically in the records of subject 9, figure 1, and of subject 4, figure 3. Failure of the hamstring group of muscles to relax fully, due to contracture, would cause that group to exert an action anatagonistic to the quadriceps group in the delivery of the knee-jerk, thus lessening the extent of the response.

In all subjects the extent of the knee-jerk decreases with the onset of fatigue. The amount of exercise necessary to produce fatigue depends on the physical condition of the subject. In some cases it was deemed advisable to discontinue the experiment on account of the effect of the exercise on the respiration and heart.

The data presented in this paper as shown graphically in figures 3 and 4 demonstrate that the characteristics of the knee-jerk as influenced by exercise conform very closely to records obtained from excised skeletal muscle under similar conditions. It seems evident that the factors influencing these characteristics in excised skeletal muscle likewise influence the intact human skeletal muscle. This corroborates and explains the work of one of the writers, Brown (1925), which showed that exercise may increase or decrease muscle tonus as exhibited by the knee-jerk.

It is evident from the control series that the phenomenon designated as "treppe" is due to exercise since the phenomenon described does not appear in the control series. In case of subjects 10, 11, 12, figure 9, there is a gradual decrease after which there is established a more or less uniformity in the average height kicked. The sharp decrease in the average height kicked by subject 2, figure 9, is due to the fact that he was conscious of the necessity for warding off sleep. This runs parallel to the results obtained by one of the writers (1924a), in investigating the effect of sleep on the tonus skeletal muscle. Subject 8 shows an increase of 5 mm. in height kicked at the beginning of the series. After a short time however this constant higher average is maintained.

The graph, figure 9A, representing the averages for the control period of the whole group shows a slight decrease early in the series after which a fairly constant height is maintained.

CONCLUSIONS

1. Intact human skeletal muscle exhibits the phenomenon of "treppe," as shown by changes in the extent of the knee-jerk under conditions which conform closely to those governing this phenomenon in excised skeletal muscle.

2. In the series of records made by certain subjects, contracture is evidenced by a transient decrease in the extent of the knee-jerk.

3. With the onset of fatigue, the extent of the knee-jerk decreases in proportion to the amount of fatigue.

4. The "treppe" exhibited in intact human skeletal muscle, as shown in the initial increase in the extent of the knee-jerk, is probably caused by the accumulation of small amounts of the commonly recognized fatigue products: carbon dioxide, lactic acid and manopotassium phosphate.

5. The final decrease in the extent of the knee-jerk is probably due to the accumulation of excess amounts of lactic acid and other fatigue products.

6. The phenomenon designated as "treppe," contracture, and fatigue in intact human skeletal is due to exercise as shown by a control series from which exercise was omitted.

BIBLIOGRAPHY

BOWDITCH, P. 1871. Berich. d. mathem-phys., xxiii, 652.
BROWN, L. T. 1925. This Journal, lxxii, 241.
GRUBER, C. M. 1923. This Journal, lxiii, 338.
LEE, F. S. 1907. This Journal, xviii, 267.
RANKE, J. 1865. Tetanus, Leipzig.
TUTTLE, W. W. 1924. This Journal, lxviii, 338.
1924a. This Journal, lxviii, 345.

THE EFFECTS OF CAFFEINE AND THEOBROMINE UPON THE FORMATION AND EXCRETION OF URIC ACID[1]

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Both caffeine and theobromine are consumed, in a large measure, in our common beverages (coffee, tea, cocoa, chocolate, etc.). During the past eleven years the average per capita consumption of coffee in the United States has been 11.6 pounds (a minimum of 8.9 pounds in 1913 and a maximum of 12.8 pounds in 1920,—reports of C. E. Bickford & Company, coffee brokers, San Francisco, California) and approximately one pound of tea. Assuming that the average caffeine content of coffee be 1.5 per cent and that of tea 2.5 per cent, this would give an annual per capita consumption of approximately 90 grams, or 225 milligrams of caffeine per day. This per capita consumption is misleading because many children and non-users of tea and coffee have been included in the figures used. Moreover only two sources of caffeine and other methylated xanthine compounds (theobromine, theophylline) have been considered. In addition to their ingestion in beverages both caffeine and theobromine are used extensively in therapeutics.

There has been considerable dispute as to the effect of these substances upon the formation and excretion of uric acid. The work thus far has been, for the most part, limited to a study of urinary excretion. The probable association of residual uric acid with such clinical manifestations as gout, arthritis and urinary calculi emphasizes the importance of a simultaneous study of its concentration in the blood and the urinary excretion.[4]

It has been suggested that the effects of caffeine and theobromine might

[1] Aided by a grant from the Research Board of the University of California.

[2] Part of the work presented in this paper is taken from a thesis submitted by Alfred A. de Lorimier in partial fulfilment of the requirements for the degree of Master of Science in the Graduate School of the University of California.

[3] A preliminary notice of this work appeared in the abstracts of the Journ. Pharm. Exper. Therap., 1925, xxv, 167.

[4] Even a study of this nature neglects the consideration of the excretion of uric acid in the sweat and by way of the intestinal tract.

vary after their prolonged ingestion. Therefore the experiments reported in this paper have been extended over a long period as outlined below. Since it is a well-known fact that man, in contrast to experimental animals, possesses (at the most) a low power of destruction of uric acid, we have used human subjects for this work. These were healthy men picked from a list of fifty or more volunteers amongst the inmates of the prison at San Quentin. These men were placed on the following "low purine" diet.

ARTICLES	DAILY ALLOWANCE
Milk (raw)	500 cc.
Cheese	50 grams
Butter	60 grams
Jelly	30 grams
Raisins (seeded)	25 grams
Tomatoes (canned)	40 grams
Shredded wheat	60 grams approximately (2 biscuits)
Graham crackers	100 grams
Bread (Graham)	200 grams approximately
Potatoes	200 grams
Sugar	50 grams
Hard candy (pure cane sugar)	30 grams
Postum	25 grams (powdered substance)
Sodium chloride	4–5 grams (as desired)
Rice	75 grams (dry weight— three times per week

Although the individual apportionments of these substances varied to a slight extent they were kept constant for each subject throughout the experimental period.

PLAN OF EXPERIMENTS. *Part A.* During the first set of experiments observations were made upon four subjects for a period of ten weeks.

1. A preliminary period of 5 days was allowed to accustom the men to the new diet and thereby to judge the quantities of each food which were to be maintained constant throughout the ensuing periods.
2. Control period, 7 days (low purine diet).
3. Caffeine period, 10 days. (Same diet as above with the addition of oral doses of caffeine, varying from 0.25 to 1.0 gram per day.)
4. Control period, 11 days. (Low purine diet.)
5. High protein, high purine diet. 3 days. (Liver ingestion.)[5]
6. Control period, 7 days. (Low purine diet.)

[5] Followed by four days on a generous meat diet, during which time no data were obtained.

7. Theobromine period, 7 days. (Same diet as above with the addition of oral doses of theobromine, varying from 0.5 to 1.75 grams per day.)

8. Control period, 8 days. (Low purine diet.)

9. Theobromine period, 3 days. (Regular low purine diet to which the more highly soluble theobromine salts (acetate, salicylate) were given orally in doses from 1.5 to 2.0 grams per day.)[6]

Part B. The diet was essentially that described under part A: three subjects were used, only one of whom (J. W.) went through the part A experiments. In this second set of experiments, which lasted about two weeks, uric determinations were made at frequent intervals during the day.

1. Control period, 7 days. (Low purine diet.)

2. Control day, with frequent blood and urinary determinations.

3. Caffeine day. (Same diet as above with the addition of 750 mgm. of caffeine at breakfast and the same routine of analyses.)

4. Control period, 3 days. (Low purine diet as above.)

5. Control day with frequent blood and urine determinations.

6. Theobromine and caffeine day. (Same diet as above with the addition at breakfast of 1950 mgm. of theobromine sodium salicylate for subjects F. G. and J. W., and 1000 mgm. of caffeine for subject C. M. The regular routine of analyses.)

7. "Follow-up" day. Urinary analyses only. (Regular diet.)

METHODS. *Part A.* The blood samples were taken before breakfast twice a week by ordinary venepuncture, using lithium oxalate as the anticoagulant. Within ten minutes of the time the sample was obtained the blood was deproteinized by the Folin-Wu (1) method. The uric acid determinations were made on these blood filtrates within six hours, by Benedict's (2) method.

Urine samples were collected over 24-hour periods. An excess of chloroform was kept in the collecting bottles and storage containers to prevent bacterial decomposition. Uric acid was determined by the Benedict and Franke (3) method within four hours after the 24-hour sample was complete.

Part B. The analytical methods and the general procedure were the same as in A except that the blood and urine samples were obtained in two and three hour periods, as outlined in charts 9, 10, 11.

DISCUSSION. Before discussing the actual experimental results presented in this paper let us consider briefly certain variables which might modify the physiological action of these drugs. In the first place caffeine and theobromine were given orally in our experiments and it is therefore impossible to estimate either the rate or the total amount absorbed. Ab-

[6] These highly soluble theobromine compounds (sodium theobromine acetate and salicylate) were given in order to check up the absorption of the less soluble theobromine base. No difference in action was observed.

sorption is dependent not only upon the cellular activity of the mucosae, but also upon such factors as the bulk of the food residues, bacterial activity, peristaltic rate, etc., all of which are affected by energy output (work, rest, temperature). Secondly, the same factors which influence absorption of a drug also play a part in the rate of excretion (by way of the urine, perspiration or feces). A third important factor is that of tolerance. In one instance we may be dealing with an individual who has acquired a tolerance by long use of caffeine or theobromine beverages and who would therefore give a less intense or a more prolonged reaction than the individual who had received the drug for the first time. Finally, there is the probability that varying amounts of uric acid undergo decomposition in the blood and tissues.[7] The numerous environmental factors mentioned above might also play a part here.

The environmental and energy factors were perhaps the most constant in our subject, E.B., who was a librarian. A glance at chart 1 shows that the first dose of caffeine, 250 mgm., caused a marked initial increase in the excretion of uric acid (approximately 35 per cent above the average, or 23 per cent more than the highest value of the 10 day control period). This immediate rise was followed by a gradual decrease until a second stimulating effect was obtained by a dose of 500 mgm., which in turn was followed by a similar decrease and a third rise when caffeine was increased to 750 mgm. In this connection Mendel and Wardel (6) state: "The increase in the excretion of uric acid after adding coffee, tea or caffeine to a purine free diet seems to be proportional to the quantity of caffeine ingested." Data presented in charts 1 to 4 do not indicate a definite proportionality. Regardless of this increased urinary excretion there was also an increase in the uric acid content of the blood. This total increase in uric acid formation might have been brought about by:

1. Increased formation by oxidation of the caffeine (or its partially demethylated derivatives).

2. Increased nuclear activity, associated with a general stimulation of metabolism.

3. Either or both of the above, together with a non-compensating elimination.

[7] This question of the enzymic destruction of uric acid is much disputed. Folin, Berglund and Derick (4) state that: "The unique and characteristic high levels of uric acid in normal human blood are due to a lack of responsiveness on the part of the human kidney," and—"the uric acid destruction stops the instant the blood is removed from the living animal" (dog). Recent experiments lead Bollman, Mann and Magath (5) to think that: "The destruction of uric acid in the normal dog depends on the presence of the liver, as this process ceases as soon as the liver is removed." Some unpublished work by ourselves indicates that there is a 19 to 33 per cent destruction of uric acid, as determined by the direct colorimetric method, when sterile blood is kept at body temperature for a period of 2 to 8 days.

CHART 1.

Subject "E.B." Age 28 Weight 162 lbs.

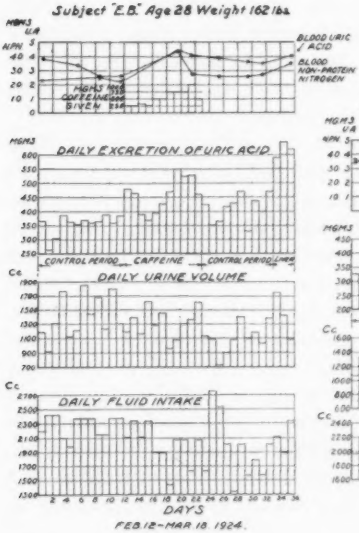

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CHART 2.

Subject "H.R." Age 36. Weight 152

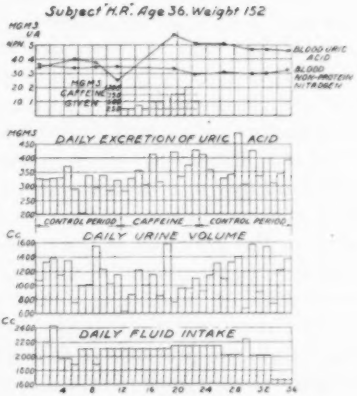

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CHART 3.

Subject "H.W." Age 44. Weight 167 lbs.

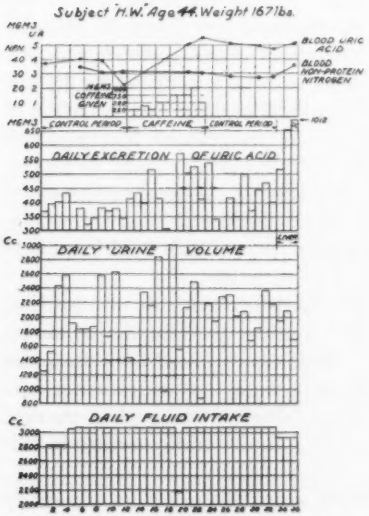

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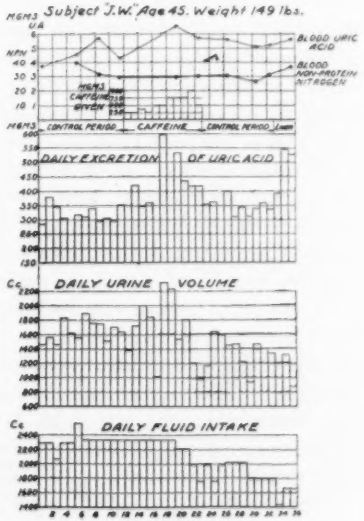
CHART 4.

Subject "J.W." Age 45. Weight 149 lbs.



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First let us consider the possibility of the increased formation of uric acid from caffeine and theobromine. Mendel and Wardell (6) state that: "The increase in the amount of uric acid excreted under these conditions (using "Kaffee Hag," caffeine and tea) is equal to the quantity of uric acid which would be obtained by the demethylation and subsequent oxidadation of 10 to 15 per cent of the ingested caffeine."

It will be recalled that the structure of the caffeine molecule differs from that of theobromine only by having the additional methyl group in position one. From the work of Krüger and his associates (7) it is probable that the methyl groups in the xanthine ring are decreasingly stable in the order 7, 1, 3. The methyl group in position 7 is common to both and if the work of Krüger and his associates be correct, there would be the possibility of a common decomposition product, 7 mono-methyl xanthine. If the stages in the oxidation of these substances are similar we should expect at least an equal amount of uric acid to be formed from molecular equivalents. With an additional methyl group in caffeine one should expect an extra stage of demethylation and we might therefore anticipate even less uric acid from oxidation of the caffeine molecule. In our experiments the doses of theobromine were almost double those of caffeine and if uric acid were being formed from direct oxidation of the molecule we should have further reason to expect it to be produced in larger amounts during the period of theobromine ingestion. The data presented in charts 5, 6, 7 and 8 indicate that such was not the case and that there was actually a decrease in the urinary excretion. The slight increase which was observed in the uric acid content of the blood can be accounted for by the diminished excretion. This diminished excretion and the accumulation of uric acid in the body tissues would suggest that theobromine had produced a "depressant effect" upon renal activity but that no increased formation of uric acid had been brought about by oxidation of the theobromine molecule. With the above mentioned structural similarities it is hardly reasonable to assume that the increase in the formation of uric acid in case of caffeine could be due to direct oxidation of its molecule.

As a second possible source of the increased formation of uric acid, at least following caffeine ingestion, we must consider the stimulating effects of this drug upon cellular metabolism. It has been found by Edsall and Means (8), Means, Aub and Du Bois (9) and others, that caffeine increases the basal metabolic rate from 5 to 30 per cent. With such a marked increase in cellular activity there should be a measurable increase in uric acid production.

The results of the part B experiments in which the uric acid was determined at intervals during a 24-hour period before and after caffeine ingestion, furnish additional evidence of this stimulation of nuclear metabolism, see charts 9, 10, 11. Although the blood uric acid remained constant it is

CHART 5.

Subject "E.B."

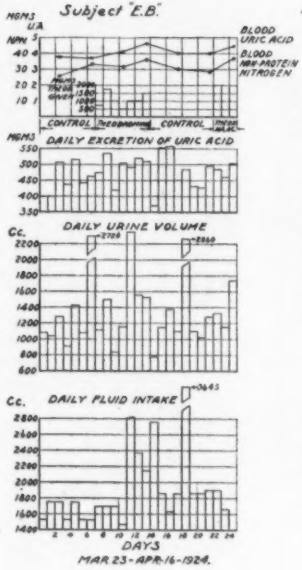

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CHART 7.

Subject "H.W."

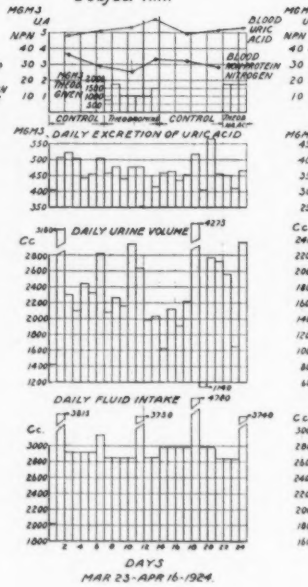

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CHART 6.

Subject "H.R."

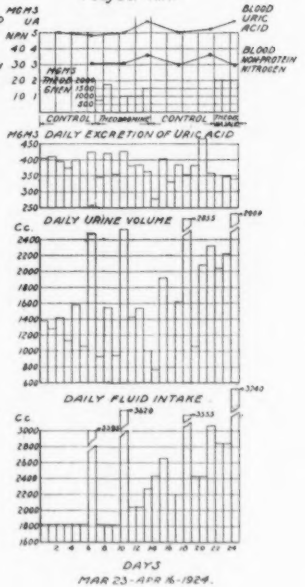

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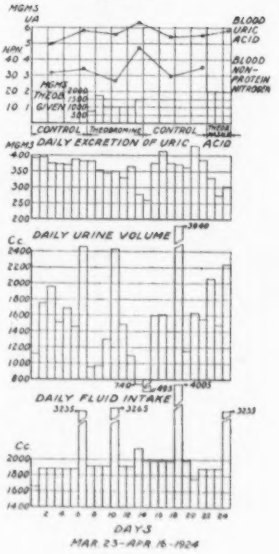
CHART 8.

Subject "J.W."



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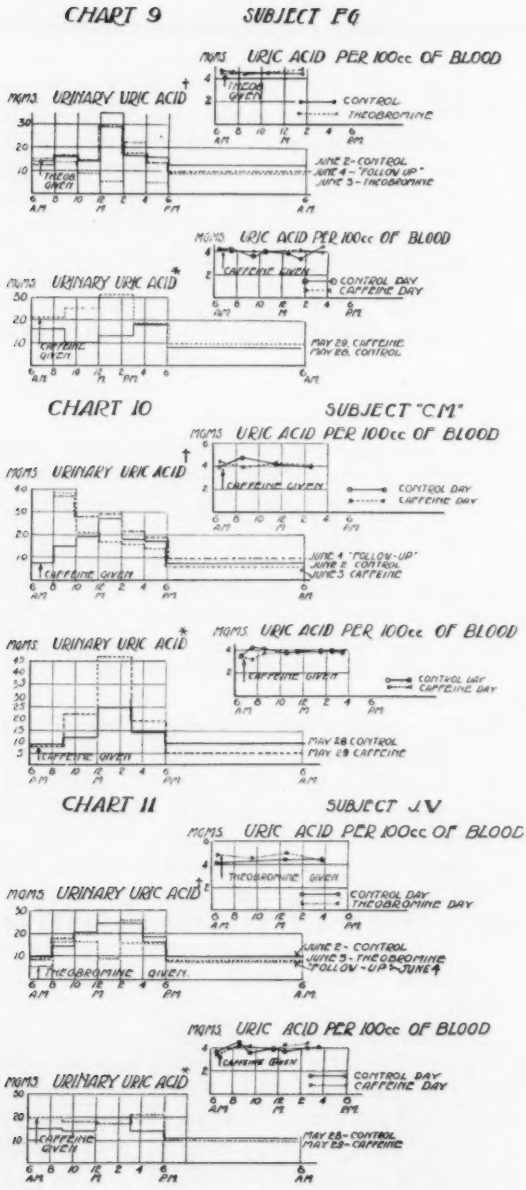

* In plotting the uric acid values, the total milligrams excreted were divided by 3, the number of hours in an experimental period (by 12 in the 6 p.m. to 6 a.m. period).

† In this set of observations the experimental period was reduced to 2 hours. In both instances the curves are drawn to represent the excretion in milligrams per hour.

evident that there was an increased excretion following the ingestion of caffeine. Let us consider subject F. G., chart 9. The uric acid excretion on the control day was 265 mgm., on the caffeine day it was 400 mgm. In case of subject C.M., chart 10, the respective values were 293 mgm. and 347 mgm.[8] In case of subject J.W., chart 11, values of 320 mgm. and 348 mgm. were obtained on the control and caffeine day respectively. As can be seen, the increased production and excretion of uric acid occurred within 8 to 12 hours, the maximum excretion being in all cases between the 5th and 7th hours. In every instance, except subject F. G. (on May 29) this initial increase in uric acid excretion was followed (during the night hours) by a decreased excretion. The theobromine results consistently showed diminished excretion.

In contrast to the apparent depressant effects[9] of the drugs very definite stimulation of uric acid excretion was obtained when the subjects were fed a high protein, high purine diet. On each of three days (34th, 35th and 36th, see charts 1, 3 and 4) three of the four subjects (E.B., H. W., J.W.) received from 200 to 225 grams of liver in addition to the regular diet. The fourth subject, H. R., did not care for liver and served as a control. In this period there was an immediate and very marked increase in the excretion of uric acid (see charts 1, 2, 3 and 4), with no significant change in the uric acid content of the blood.[10] However, the prompt increase in the excretions are indicative of there having been definite stimulation, i.e., in contrast to the effects of either caffeine or theobromine. This interpretation is in accord with the findings of Lewis, Dunn and Doisy (10) and Folin, Berglund and Derick (4).

There is the question as to whether this increased uric acid excretion has been produced by a parallel increase in the volume of urine. Morris and Rees (11) produced salt diuresis in rats and rabbits and state that: "As with urea, the output of uric acid is affected by the volume of urine excreted more than any other factor" and "There is no evidence of uric acid arising from increased kidney activity. The increased output is proportional to volume of urine excreted." On the other hand Mareš (12) reports that: "The supposed parallelism between the amount of

[8] Repeating the experiment three days later with this subject we obtained values of 299 and 382 mgm. for the control and "follow-up" days respectively and only 284 mgm. were excreted during the caffeine day, constituting the only exception. Edsall and Means (8) found a somewhat similar depression with a second dose of caffeine.

[9] Although charts 1, 5, 6, 7 and 8 indicate that there was a slight increased retention in the non-protein nitrogen of the blood, we do not feel that these results alone definitely indicate a depression but that when considered together with the excretion of uric acid, afford rather strong evidence of a depression in the excretion of organic substances.

[10] We do not mean to infer that prolonged ingestion of this high purine diet might not also have caused a greater damming back of the uric acid in the blood.

urine and the amount of uric acid excreted was not observed." Mendel and Brown (13) state that: "The incidence of large volume and increased uric acid output is accidental and by no means constant."

A study of the data obtained during the 12-day control period, prior to the administration of caffeine (see charts 1 to 4), indicates that under normal conditions[11] there is a relationship between the volume of urine and the quantity of uric acid excreted. On the other hand when the water intake, and subsequently the volume of urine excreted, was greatly increased there is absolutely no evidence of this relationship. This latter point was proven in these experiments by resorting to extreme water diuresis: subject E. B. on the 11th and 19th days, H. R. and J. W. on the 7th, 11th, 19th and 25th days, H. W. on 2nd, 12th, 19th and 25th days; see charts 5, 6, 7 and 8. As can be seen, although the fluid intake and the urine volume were greatly increased on each of these days, not one of the four subjects showed a parallel increase in the excretion of uric acid. These results suggest that uric acid is not passively excreted but that it is eliminated through specific activity of the renal cells.

Let us consider the correlation between this cellular activity, the acquisition of tolerance and the accumulation of uric acid in the blood. As mentioned, the results with caffeine (see charts 1, 2, 3 and 4) show an irregular but immediate increase in uric acid excretion following either an initial dose or a marked increase in dosage. Under the same conditions the initial dose of theobromine failed to give this immediate increase and on continuation even caused decrease in the uric acid excreted. We might reason that the observed increases in uric acid excretion, after caffeine, were caused indirectly, i.e., by the general metabolic stimulation previously mentioned, and that primarily the renal cells were reacting to the increased endogenous uric acid. Subsequently the excreting cells reacted to the foreign and more toxic substances (caffeine or various demethylated derivatives) resulting in a specific elimination of these substances to the partial exclusion of uric acid. The fact that the presence of large amounts of uric acid in the blood may stimulate the kidney to specific elimination has very recently been shown by Bollman, Mann and Magath (5). These workers found that when as much as 864 mgm. of uric acid were injected intravenously into a hepatectomized dog, 85 per cent could be recovered in the urine within 13 hours—a striking compensatory elimination, when the blood content exceeded its normal amount. The results obtained with theobromine are in accord with the above theory as to the acquisition of tolerance.

[11] Although the fluid intake was fairly constant there are many variations in the daily urine volumes. We have no measure of the water excreted through the skin, the amount of which would vary directly with external temperature, exercise, etc. The possible excretion of uric acid in the sweat is likewise an unknown factor. The relationship suggested is relative rather than absolute.

We have been unable to procure evidence to prove definitely that theobromine is devoid of any power of metabolic stimulation but our results indicate that there was at least no increased formation following its ingestion. The initial dosage seems simply to have allowed a normal excretion of uric acid. Subsequent doses apparently led to the same selective elimination of theobromine (or its methyl derivatives) and uric acid retention, just as was observed in case of caffeine.

CONCLUSIONS

1. The ingestion of either caffeine or theobromine tends to increase the concentration of uric acid in the blood.[12]

2. After caffeine ingestion there is evidence of an increase in uric acid production. After theobromine ingestion its formation seems to be unchanged, but its rate of excretion diminished. It seems probable that the increases in uric acid are not due to direct oxidation of these methylated xanthines.

3. Prolonged administration of either caffeine or theobromine seems to depress active excretion on the part of the kidney.

4. There exists no proportional relationship between the amount of drug given and the quantity of uric acid excreted.

5. Under normal conditions there exists a relationship between the volume of urine and the quantity of uric acid excreted, but under diuretic conditions (other than when produced by uric acid itself) no such relationship is evident. Apparently uric acid is actively excreted.

This work could hardly have been accomplished had it not been for the coöperation of ex-warden J. A. Johnston and the special efforts of Dr. L. L. Stanley, resident physician at the California State Prison, San Quentin.

[12] In the experimental work reported in this paper, part A was carried out February to April, 1924, and part B during May, 1925. Subsequent to the completion of the part A experiments there appeared articles by Bullmer, Eagles and Hunter (14) and by Hunter and Eagles (15) in which it was shown that whole blood contained material other than uric acid ("Substance X") which reacted with the Benedict (2) uric acid reagent to produce color. Very recently Benedict (16) has reported the presence of a second substance ("thiasine") in whole blood which also reacts with the uric acid reagent to produce color. The blood uric acid values (total color) reported in this paper are no doubt high and we are not in a position to state whether the observed changes in the uric acid of the blood were due to changes in "substance X," "thiasine" or uric acid per se. We hope to repeat a portion of this work, using an indirect method (precipitation with a silver salt) for the determination of uric acid.

BIBLIOGRAPHY

(1) FOLIN, O. AND H. WU. 1919. Journ. Biol. Chem., xxxviii, 81.
(2) BENEDICT, S. R. 1922. Journ. Biol. Chem., li, 187.
(3) BENEDICT, S. R. AND E. FRANKE. 1922. Journ. Biol. Chem., lii, 387.
(4) FOLIN, O., H. BERGLUND AND C. DERICK. 1924. Journ. Biol. Chem., lx, 361.
(5) BOLLMAN, J. L., F. C. MANN AND T. B. MAGATH. 1925. This Journal, lxxii, 629.
(6) MENDEL, L.B. AND E. L. WARDELL. 1917. Journ. Amer. Med. Assoc., lxviii, 1805.
(7) KRÜGER, M. AND G. SALOMON. 1898–9. Zeitschr. f. physiol. Chem., xxvi, 350. KRÜGER, M. AND P. SCHMIDT. 1899. Ber. d. deutsch. chem. Gesellsch., xxxii, 2677.
(8) EDSALL, D. D. AND J. H. MEANS. 1914. Arch. Int. Med., xiv, 897.
(9) MEANS, J. H., J. C. AUB AND E. F. DUBOIS. 1917. Arch. Int. Med., xix, 832.
(10) LEWIS, H. B., M. S. DUNN AND E. A. DOISY. 1918. Journ. Biol. Chem., xxxvi, 9.
(11) MORRIS, J. L. AND H. M. REES. 1923. This Journal, lxvi, 363.
(12) MAREŠ, F. 1910. Pflüger's Arch., cxxxiv, 59.
(13) MENDEL, L. B. AND E. W. BROWN. 1907. Journ. Amer. Med. Assoc., xlix, 896.
(14) BULLMER, F. M. R., H. A. EAGLES AND G. HUNTER. 1925. Journ. Biol. Chem., lxiii, 17.
(15) HUNTER, G. AND B. A. EAGLES. 1925. Journ. Biol. Chem., lxv, 623.
(16) BENEDICT, S. R., E. B. NEWTON AND J. A. BEHRE. 1926. Journ. Biol. Chem., lxvii, 267.

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The main purpose of PHYSIOLOGICAL REVIEWS is to furnish a means whereby those interested in the physiological sciences may keep in touch with contemporary research. The literature, as every worker knows, is so extensive and scattered that even the specialist may fail to maintain contact with the advance along different lines of his subject. The obvious method of meeting such a situation is to provide articles from time to time in which the more recent literature is compared and summarized. The abstract journals render valuable assistance by condensing and classifying the literature of individual papers, but their function does not extend to a comparative analysis of results and methods. Publications such as the Ergebnisse der Physiologie, the Harvey Lectures, etc., that attempt this latter task, have been so helpful as to encourage the belief that a further enlargement of such agencies will be welcomed by all workers. It is proposed, therefore, to establish a journal in which there will be published a series of short but comprehensive articles dealing with the recent literature in Physiology, using this term in a broad sense to include Bio-chemistry, Bio-physics, Experimental Pharmacology and Experimental Pathology.

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